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INTRAMURAL ANNUAL REPORT

NATIONAL INSTITUTE ON AGING

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Annual Report of the Office of Scientific Director
National Institute on Aging

The Scientific Director of the NIA is responsible for the overall direction and quality of research conducted by the Intramural Research Program (IRP) which includes nine laboratories and branches in Baltimore and the Laboratory of Neurosciences located in the NIH Clinical Center in Bethesda. The Office of the Scientific Director oversees the central administrative and support activities necessary for the successful operation of the Intramural Program. These activities are carried out by the Administrative, Procurement, and Information Offices, and the intramural program Personnel Office.

During Fiscal Year 1992, Dr. Vilhelm A. Bohr, joined the staff as chief of the Laboratory of Molecular Genetics. Dr. Bohr has extensive experience studying DNA damage and repair, and most recently served as medical officer and senior investigator for the Laboratory of Molecular Pharmacology in the Developmental Therapeutics Program of NCI's Division of Cancer Treatment. He is particularly known for his work that demonstrated significant differences in the rates at which active genes and protooncogenes, as compared to nontransmitted regions of the genome are repaired. Specific deficits in DNA repair are present in certain heritable diseases (xeroderma pigmentosum, Li-Fraumeni syndrome) which are linked to an increased risk of cancer. Similar studies will be directed toward age-associated alterations in the genome.

Two important initiatives were launched this year by the Baltimore Longitudinal Study of Aging (BLSA) in cooperation with the Laboratory of Clinical Physiology and the Laboratory of Cardio--vascular Science. The BLSA Vascular Initiative involves a multidisciplinary set of studies examining age, race, and gender differences in blood pressure, arterial stiffness, and thickening of the heart muscle, and the relationships between these parameters. Particular interest will be focused on the differences between African-American and Caucasian men and women in such variables as age-related increases in blood pressure and prevalence of hypertension. The BLSA Perimenopausal Study will examine physiological and psychological changes occurring before, during and after menopause. Since there are racial differences in some aspects of menopause, sufficient numbers of African-American and Caucasian women, aged 45 to 55 years, are being recruited for this study.

The National Center for Nursing Research and the National Institute on Aging signed an agreement to develop a joint program in geriatric nursing research at the Gerontology Research Center.

Research activities of the eight laboratories and two branches of the IRP as well as the Epidemiology, Demography and Biometry Program are fully reported in this annual report, however, a few highlights are summarized below.

Atherosclerosis and Aging. Ongoing research in the Laboratory of Cardiovascular Science suggests that changes in the state of differentiation of vascular smooth muscle cells, which normally provide physiological tone, may contribute significantly to age-associated increases in the susceptibility to stroke and other vascular diseases. This research shows that smooth muscle cells from older animals, as well as hypertensive animals dedifferentiate, degrade the vascular matrix and migrate to the vessel lumen. Also, these older cells fail to properly regulate the expression of a critical nuclear regulatory molecule involved in the switch from contracting to proliferating smooth muscle cells. This work identifies changes in the fundamental behavior of smooth muscle cells from aging animals which may underlie age-associated increases in vascular disease.

Early Markers of Alzheimer's Disease. Laboratory of Personality and Cognition examinations of Baltimore Longitudinal Study of Aging (BLSA) participants have demonstrated that individual differences in cognitive performance on the Mini-Mental State Examination can be predicted up to 9-15 years later by performances on the Benton Visual Retention Test (BVRT), a nonverbal test of memory. This finding is useful since mental status tests are often used to screen and evaluate people for significant cognitive impairment and dementia. Extensive analyses of test results revealed that the predictive power of BVRT scores was independent of age effects and intelligence, both of which are associated with BVRT performance. In addition, immediate and short-term memory task performance and scores from continuous memory tasks for words and numbers were predictive of mental status among BLSA men and women.

Alzheimer's Disease, Brain Metabolism and Responses to Stimulation and Drug Treatment. Advances in Positron Emission Tomography now allow rapid assessment of brain region responses following cognitive stimulation and drug treatment. Studies of cognitively stimulated patients with Alzheimer's disease show comparable responses to those seen in normal volunteers. Further, the continuous infusion of arecoline over a three week period to 19 demented, elderly subjects induced a significant improvement in memory. These studies suggest that such drugs, including those under development in the Laboratory of Neurosciences, may find use in patients with moderate deficits.

Hypertension/Brain Damage and Dysfunction. Recent research in the Laboratory of Neurosciences on patients with controlled hypertension have shown evidence for hypometabolism in 13 regions of the brain clustered around major arterial supplies.

These studies suggest that long standing hypertension, even when treated, can cause significant subclinical brain dysfunction.

Developing Cognitive Enhancement Drugs. In collaboration with the Laboratory of Neurosciences, scientists in the Laboratory of Cellular and Molecular Biology are analyzing the effects of novel cholinergic drugs on learning performance in rats and mice. They tested the effects of a potent anticholinesterase, phenylphysostigmine, for reversing learning impairment induced by scopolamine, a muscarinic antagonist. Rats were tested in the 14-unit T-maze and mice were tested in a Morris swim maze. In the animals tested, the anticholinesterase had a prolonged action and reduced the learning impairment. Other compounds developed in the NIA Laboratory of Neurosciences had an even wider range of therapeutic efficacy with minimal side effects.

Selective Attention in Healthy Aging and in DAT. NIA neuroscientists studying selected and divided attention to auditory and visual stimuli found that aging was not associated with changes in the ability to attend selectively to one sensory channel, yet increasing difficulty was noted while shifting attention from one sensory channel to another. By contrast, patients with dementia of the Alzheimer's type had impairment of both selective and shifting attention, although the impairment of shifting attention was more marked.

Treating Brain Tumors with Water Soluble Drugs. The alkylating anticancer agent, chlorambucil, which enters the brain only slowly, and usually is ineffective against brain tumors, was made more effective by converting it into a tertiary butyl ester. This resulted in rapid brain uptake and a long plasma half-life, while retaining significant antitumor activity. The Laboratory of Neurosciences team also found it to be active against human brain tumors previously insensitive to chlorambucil. This product is entering clinical testing. Thus, water soluble drugs can be appropriately modified to increase brain uptake and half-life in plasma and be used to treat central nervous system diseases.

Growth Hormones and Aging. Investigations into the relationship between growth hormone (GH) deficiency and aging have shown reductions in pulsated GH secretions and serum insulin growth factor 1 (IGF1) levels in healthy older men. However, Endocrinology Section scientists found that normal production was restored after older subjects received injections of growth hormone releasing hormone (GHRH) daily. This research is significant because growth hormone deficiency is associated with muscle and bone wasting and a reduction of strength. An initiative designed to treat frailty is being organized.

Model to Study Bone Replenishment. Scientists in the Laboratory of Biological Chemistry developed a rat model which they are using to screen alternative therapies to estrogen and growth hormone treatments which are currently used to replenish bone.

The NIA researchers have noted that growth factors infused into the femur can increase the expression of bone specific genes of old rats. Preliminary findings show that a collagenase inhibitor which localizes to bone induces dramatic effects on ovariectomized rats where the resorption of bone is supposedly maximal.

Aging and Cancer. Researchers have developed several animal models to study the changes in cancer incidence and growth with age. In addition to the nude mouse model developed to grow human prostate and small lung carcinoma cells, Laboratory of Biological Chemistry investigators have isolated spontaneous breast tumors from aged rats which they are assessing for genetic alterations associated with aging and tumorigenesis. This work is significant because post-menopausal breast cancer is common in elderly women, but previously there have been no animal models to study spontaneous, age-onset mammary tumors.

HIV and the Older Patients. The age of an HIV infected person has been shown to have a powerful influence on the progression of an HIV infection to frank AIDS. Recent research in the Clinical Immunology Section shows age-related alterations in host responses with augmentation of the release of inflammatory factors. In older HIV infected patients, there is a significant increase in the rate of helper T cell loss which may be due to a decline in the body's ability to replace HIV destroyed T cells.

Improving Continence in Nursing Homes. Laboratory of Behavioral Sciences investigators continued their studies on how to improve the treatment of incontinence in nursing home residents. Work completed this year showed that prompted voiding intervention could be implemented and handled by the on-site nursing staff in a community-based nursing home. Once staff were trained to implement and supervise the prompted voiding treatment they were able to increase dryness in study patients from 45 to 64 percent, and maintain this level over the 12-week study period.

Intramural Research Information Services

Comprehensive Information Program. Two news releases initiated in the IRP Information Office got wide coverage both locally and nationally. The first on hearing and gender differences was used by the Associated Press and appeared on the front page of *USA Today* and in many other papers. Prostate specific antigen testing was the second release, and it was featured in the *New York Times, Washington Post, Baltimore Sun,* Washington and Baltimore area TV stations, and on the wire services.

Special report highlights for Congress included one on a new model for studying cancer, and another on lowering incontinence in nursing home patients. Briefing material on menopause studies was prepared for Dr. Healy in connection with an interview by an author writing a book on women's health.

Three status reports for the National Advisory Council on Aging were prepared, as were an introduction to the NIH research fellows catalog, material for the NIH Disease Prevention and Health Promotion Report, the FY91 International Activities Report, and an update of the IRP Fact Sheet. A fact sheet was prepared on the BLSA and its need to recruit women for menopausal studies and African-Americans for a number of studies of gender and race differences. The latter was supplied to NIA in Bethesda for a BLSA recruitment campaign and sent to local colleges, newspapers, and senior newsletters resulting in articles in the *Johns Hopkins Gazette, Baltimore Jewish Times, Senior Digest, Catholic Review, Coppin State Alumni Newsletter,* and *Pennysaver* publications. Nationally, the office worked with the *Chicago Tribune* on an 8-part series on aging published in January and then as a paperback book, *Aging on Hold,* which features a number of Institute grantees and intramural researchers. National TV broadcasts on PBS, ABC-TV, Home Box Office, and the cable Family Channel also used interviews and footage arranged by this office.

Educational and Professional Activities. The IO coordinated a three day visit by a University of Hawaii group developing a telecourse on aging, also to be broadcast over PBS stations; many NIA scientists were interviewed. Staff made presentations and gave tours or briefings to a variety of publics addressing some 240 individuals overall; staffed an NIA exhibit at the Maryland Gerontological Association Conference and for NIH at the President's Committee for Employment of People with Disabilities annual meeting; provided NIA handouts for the latter exhibit, as well as for the Baltimore FEB Federal Women's Conference and FEB Disability Awareness Conference.

Technical Development Section

This year,we replaced almost all of the hardware of the old central computer system; much of which was 10 years old. The new hardware is technologically modern, as reflected by the reduced cost (both acquisition and maintenance costs), higher performance, smaller space requirements, and lower power consumption. Approximately 500 sq. ft. of the computer room was converted to general office space. The central VAX system is still extremely active, and maintains accounts for over 200 users, distributed among the nine laboratories within the GRC. While the preponderance of activity on the system still relates to data collected in the laboratories, i.e., data entry, maintenance of databases, statistical analysis, its usage as the hub of the building-wide network has grown from 43 nodes last year to over 110 at the present time. By running Dec's PCSA network software, we have virtually eliminated the need for local LANs. We have just connected our network to the InterNet network, which will allow access to NuNet at the Bethesda campus, E-Mail, file transfer, as well as access to all major network services world-wide. We will soon be giving classes to the GRC staff, on accessing the services available there.

The installation of the GRC telephone system was completed and became operational. The implementation of this system should result in significantly lower telephone charges, that will be further reduced when the FTS dial tone is brought directly to GRC. Since the installation, there has been considerable movement of laboratories and offices within the GRC. This would have created significant problems and delays with the moving of the old telephones; however, we have been able to fulfill 98% of the requests for changes to phone service - without outside assistance.

Several PC-based laboratory systems were completed in the last year. A major effort of the TDS was the development of software for the Poolesville Diet Restriction Study or the GRC Molecular Physiology and Genetics Section. This software consists of a database and data entry and retrieval programs. The VAX-based database contains over 100 different measurements, taken from over 100 monkeys, typically four times a year beginning in 1987. A series of programs were written to reduce the effort and increase the accuracy of the data entry. PC-based retrieval software, tightly connected to the VAXs, allow easily-configured subsets of the data to graphically checked, placed in Excel spreadsheets or converted to SPSS data files. Most of the software and hardware is currently functional.

A continuing effort was given to the expansion of a PC-based system, to collect calcium and electrophysiological data for the Laboratory of Cardiovascular Sciences. The system includes seven PC's with locally developed and fabricated interface cards all connected, via ethernet, to a VAX cluster. Additional PC cards, that will digitally integrate the 4 microsecond wide fluorescence, are currently being developed. In the PC's, menu-driven software allow the experimenter to setup a wide variety of experiments and collect relatively large quantities of data, that are both shipped immediately over the network to the VAX's and stored locally. VAX-based software immediately constructs a graphical summary and performs a set of predefined calculations, which are fed back to the experimenter, and places the verified data into a storage structure. Later, using "friendly" PC-bases programs, the data may be retrieved from the structure and downloaded into popular software packages, such as Lotus 123, Excel, CricketGraph and Adobe Illustrator. Interactive, windows-based software was developed for analyzing electro-physiological portions of the data. The large amounts of data being collected (13,000 files, 500 MegaBytes) necessitated the development of a multi-volume data storage scheme and an automated backup system.

Recently, the arrival of Dr. Bohr required an analysis of the new requirements for the Laboratory of Molecular Genetics (LMG). It was determined that adding his Molelular Dynamics Scanner and Densitometer to the GRC network would allow the transferring of 40 MegaByte TIF files to servers located elsewhere within LMG. This will free the scanner and densitometer for maximum number of scans per day, while allowing investigators, at workstations, to access previously collected scans for analysis. We are now developing the archiving scheme for maintaining their database.

Photography and Arts Unit

In addition to the normal production of negatives, slides, prints, and poster materials, the computer graphics system was expanded to include the development of new software for use in the Unit. This new software enables the Unit to produce graphs which closely match almost any type of variation presented to the Unit. Plans are being made to make this software available to any VAX user with the proper hardware. In addition, new hardware has been ordered for the VAX machine room, for use by anyone in the building.

An increased demand for service this past year was matched by an increased capacity from the computer graphics system. A considerable amount of time was saved, not only because some of the work was done by others outside the Unit, but also because the time expended on the computer is far less than the time needed for conventional processes.

The MacIntosh continues to help the Unit add to the computer graphic's capability. In addition, a color thermal dye transfer printer has been added this year, to increase the quality of work being performed by this department.

Library Unit

The GRC Library continued a successful service year in 1992. Besides routine operations, several areas and projects are worthy of discussion. The statistics of Medline search has increased from 1,020 in FY '91 to 1,400 in FY '92. This increase of 37% indicated a need for another Medline search facility. A Dec-Station, 325c PC was purchased. However, funds for subscriptions of additional copies of the Medline on compact disc was unavailable. The new computer, nevertheless, will enable us to apply the window version of the system's searching software, which is to be released in August 1992.

Directly resulting from bibliographic searchings, the GRC intramural reserchers made 62% (or 980) more document delivery requests this year. The library staff has successfully obtained 2,571 articles and books for in-house researchers, and filled an additional 342 requests for outside institutions. When the two numbers are added up, FY '92 interlibrary loan service reaches a record high of 2,913 transactions. It can be translated into a 169% productivity, as compared with the FY '91 record. Compared to the 1990 interlibrary loan data, the FY '92 data amounts to 223% increase. Interlibrary loans has thus become the library's fastest growing operation.

The distribution of supplying sources for our inter-library loan requests has changed. We used to depend on the NIH Library for providing nearly 85-90% of our requests. This year, only 60% came from their area. This is due to the policy change made by the NIH Library. The initial policy change was unfavorable to the GRC non-permanent employees. After negotiations, the NIH Library agreed to provide us with the materials, which they are capable of supplying; however, no longer will the NIH Library Staff continue to photocopy our needed items at the adjacent National Library of Medicine (NLM). Therefore, our requests, which are directly being sent to the NLM, has increased by 27%. The University of Maryland at Baltimore County became our major provider, accounting for 9% of the total figure.

Subscription to the abstract version of ***Current Contents on Diskettes*** was started in 1992. Its weekly, compressed, data requires rather large storage space on our computers. Our IBM M30/286 machine, which is currently being used, has proved itself insufficient - both, in storage area and in performance. A Dec-Station was purchased to replace the IBM.

The librarian taught the following instructional sessions to library users: Comprehensive Orientations, Searching Current Contents on Diskettes, and Searching Medline Data Base.

Special funding was allocated this year to convert journals on microfilm. Six titles of journals, including 249 printed volumes, were replaced by microfilm cartridges. A total of 409 volumes of journals were bound in FY '92.
The special project of updating our journal holdings list is in progress. It is scheduled to be complete by the end of summer.

Animal Resources Section

During the past year, the Animal Resources Section reorganized the majority of its staff, by properly reclassifying their positions. Many of the animal care staff have been reclassified into the GS biological series, due to their involvement with investigator's projects, while still performing their animal care assignments. Their scope of work has been broadened to add a career ladder and provide the much needed technical assistance to GRC investigators.

Collaborative efforts of the ARS staff continues to increase along with program needs. Several investigator breeding colonies have been established, or revitalized, through ARS involvement. The ARS has co-authored 6 papers accepted for publication this past year.

The ARS has begun a series of investigator training classes, with a hands-on approach, to teach investigators and their technicians the skills needed to handle and manipulate animals properly, safely, and humanely. Animal technician training has continued, resulting in several technicians being certified at the national level.

Full accreditation of our animal care and use program, by the American Association for Accreditation of Laboratory Animal Care (AAALAC), continues for the 15th consecutive year. In conjunction with an AAALAC recommendation, our aseptic surgical suite has been relocated and rebuilt to meet the specifications outlined in ***The Guide for the Care and Use of Laboratory Animals***.

Population inventories and daily census show a sharp increase in animal housing and use, during the last year. Animals, being housed in suuport of studies, has increased over 15%. 6,078 mice and rats were issued from the aging rodent colonies. Care was provided for an average daily population of 13,838 rats, 9,618 mice, 14 hamsters, 7 rabbits, 36 dogs, 20 guinea pigs, 10 monkeys, and 2 pigs.

In addition to these stock animals, approximately 13,753 rats, 7,423 mice, 145 guinea pigs, 104 rabbits, 86 hamsters, 40 gerbils, 18 pigs, 6 monkeys, and 2 dogs were received, housed, and cared for by the ARS.

Nine ARS employees achieved outstanding EPMS ratings and nine ARS employees achieved excellent EPMS ratings for the year 1991. A group award was presented to the ARS staff for their dedication in helping the NIA-GRC maintain accreditation with AAALAC.

Instrument Design and Fabrication Section

In the past year, along with many 30 minute to 2 hour construction and repair jobs, the IDFS designed, fabricated, and installed equipment. The following are representative of the different jobs that were performed: installation of a safe work area, and a containment hood for investigators working with radioactive materials, constant temperature hand-warming chambers, a video camera positioning device for a mouse maze, lens holders for beam control laser equipment, a constant monitoring system for use in the swine area, and a macro analysis perfusion system for video microscopy and fluorescent measurement in isolated single cells. This past year, a new in-house telephone system was installed in GRC. The IDFS is responsible for the upkeep, repairing, and installation of new telephone lines - voice and data.

Due to the re-organization of the various GRC laboratories, the IDFS staff continues to dismantle old laboratories and set up new ones in their place. Everytime this procedures occurs, the IDFS has to relocate wiring for computer terminals. This detailed process is very intricate and time consuming. Various other projects were successfully completed.

Two IDFS employees achieved an outstanding EPMS rating and three IDFS employees achieved excellent EPMS ratings for 1991. For their efforts, these employees received individual cash awards.

ANNUAL REPORT OF THE LONGITUDINAL STUDIES BRANCH

NATIONAL INSTITUTE ON AGING

In October, 1991, the Board of Scientific Counselors (BSC) met with the Associate Scientific Director (ASD) for the Baltimore Longitudinal Study of Aging (BLSA) and the BLSA Steering Committee to determine the responses to BSC recommendations from the October 1990 review of the Longitudinal Studies Branch (LSB) and the working relationships between the BLSA director and the other laboratories whose research relates to the BLSA. The BLSA response was based on both a statement of goals and objectives for the BLSA and in part on a plan for collective leadership of the BLSA developed at the ASD's request by a group of BLSA Steering Committee members, headed by Dr. Paul Costa. Adopted in April 1991, the plan provides for three BLSA Steering Committee subcommittees: scientific directions; progress review (of ongoing research); and resource allocation (oversight of LSB and other laboratory resources devoted to BLSA activities). The statement of goals and the operating plan for collective leadership of the BLSA was enthusiastically endorsed by the Board of Scientific Counselors. The Board also agreed to our recommendation to appoint a liaison between the Board and the BLSA to monitor BLSA progress, and to determine the optimal approach for the Board's periodic review of the scientific activities, which transcend the boundaries of particular laboratories. The first liaison, Dr. Redford Williams of Duke University, met with the Steering Committee leadership in June, 1992, and regular meetings are planned for the future.

In operation for approximately 15 months as of July, 1992, the reorganized BLSA Steering Committee has resulted in a strong time commitment on the part of the members to carry out their activities. The membership of the Committee and a summary of its major activities are given in Report Z01 AG 00015-34 LSB, The Baltimore Longitudinal Study of Aging. Written reports on the ongoing activities have been received by the Progress Review Subcommittee from all investigators and oral reviews have been conducted with over a dozen investigators. The Scientific Directions Subcommittee has developed a statement of goals and objectives for the BLSA that accurately reflects ongoing research, and the Resource Allocation Subcommittee has assessed the future recruitment needs for the BLSA. The ASD is fully committed to assuring the continued success of the shared leadership concept.

The single most far reaching FY92 development in the BLSA is the commitment to implement new research initiatives relative to minority aging and women's aging. Various research possibilities in these areas had been studied for over three years. The goal was to select significant research problems that are appropriate for a longitudinal study and that take full advantage of the unique strengths of the GRC scientists that work with the BLSA.

The "vascular initiative" includes a series of interrelated projects to study racial differences between cardiovascular function and cerebral blood flow in African-American men and women and their Caucasian counterparts. The four projects comprising this initiative were developed in LCS, LSB, and LCP. On the basis of recommendations by the Steering Committee, the Scientific Director allocated additional resources for the first two listed. The names of the projects are given in the following section of the LSB Report, and the plans are described in more detail in appropriate sections of the Annual Report.

The "perimenopausal" initiative is designed to measure changes in hormone activity four times yearly in African-American and Caucasian women as they go through menopause and to follow the consequences of the observed changes for age associated losses in bone density, strength, changes in body composition as well as development of cardiovascular outcomes and metabolic diseases. The initiative originated in two sections of LCP and over the course of the study, other laboratories will be involved. On the basis of recommendations by the BLSA Steering Committee, the Scientific Director allocated new resources to this initiative.

Results of a survey of research plans of BLSA investigators by the Resource Allocation Subcommittee indicate that many ongoing studies have generated hypotheses related to potential racial differences in existing studies. Examples include studies of strength, prostate growth, age changes in sensory function, particularly vision, metabolism, body composition, nutrition and other life style variables such as smoking and activity levels, coping styles, pulmonary function, and others.

About three percent of the current BLSA participants are of African-American descent, not nearly enough for the new initiatives. Accordingly, a substantial recruitment effort has been launched to implement the two initiatives. For example, the perimenopausal initiative requires the recruitment of almost 200 African-American and Caucasian women between the ages of 45 and 55 who are currently menstruating, are nonsmokers, not taking hormone replacement therapy, do not have thyroid disease, and meet requirements for cardiovascular health. The vascular initiative requires the recruitment of 140 African American men and women equally spread over the age range from the 20s through the 80s who are initially healthy by cardiovascular criteria. With some exceptions there are sufficient numbers of Caucasian men and women in the Study who meet the health requirements of the vascular initiative.

Implementation of the two new initiatives has created new challenges for many GRC scientists who work with the BLSA and for all LSB staff who deal most directly with the increased workload in the BLSA and the necessity to recruit new participants. For several years the recruitment needs of the study were met by enrolling persons from a large waiting list, most of whom were referred by existing participants. Several mechanisms for recruiting African American participants have been implemented and it is anticipated that a contract mechanism will be used in FY 1993 to increase the number of potential volunteers. The additional workload required to schedule and manage participant visits is being addressed in part by implementation of a computer-aided scheduling and room booking system that provides a flexible, easily accessed data base that allows several staff persons to work on scheduling on a part-time basis or at different locations at the same time. Further details are given in Reports AG Z01 00624-03, BLSA Population Dynamics, and AG Z01 00625-03 BLSA Data Management.

The role of the LSB in the BLSA is to operate and manage the Study and to perform research with the Study using both historical and currently collected data. Highlights of these activities follow.

POPULATION DYNAMICS

The number of active BLSA participants is larger than ever: 682 men and 465 women.

The second follow-up of inactive BLSA participants via telephone interview began in February, 1992; over 50% of the inactive participants have been interviewed.

DATA MANAGEMENT

Installation of a new computer system for the BLSA.

Development of a computer-aided system of scheduling and booking rooms for BLSA participants.

CLINICAL EVALUATION

Physical Functioning Inventory implemented in inactive and active participants.

First true interval medical history under development.

RESEARCH: SENSATION AND PERCEPTION

Vision--First survey of self-reported problems in driving published.

Vision--First correlations between self-reported problems in visual functioning and assessments of contrast sensitivity reported.

Vision--Low levels of serum Vitamin E status found to be a risk factor for age-related nuclear opacities and maculopathy.

Hearing--Expanded BLSA hearing assessment implemented.

Hearing--First report of sex differences in age changes in hearing thresholds indicates women's hearing loss with age is about half that of men.

HEALTH/DISEASE RELATIONSHIPS IN AGING

New BLSA strength assessment procedure implemented.

Self-reported activities analyzed reveal complex sex, age and secular changes in patterns of discretionary, obligatory and personal care activities.

Time course from development of symptoms to prostatectomy estimated from BLSA medical records.

How serial measurements of prostate specific antigen provide early detection of prostate cancer published; the study is now being extended to all BLSA males over 40.

Normal decline of pulmonary function with aging tracks a constant percentile in healthy, nonsmoking men and women.

Development of ischemic heart disease (i.e., ECG abnormalities, definite angina or previous myocardial infarction) is associated with initial pulmonary impairment or a greater decline in pulmonary function than expected on the basis of aging.

STATISTICAL SCIENCES

Mixed effects models applied to nursing home outcomes--first use of these techniques to analyze health care delivery issues.

Mixed effects models applied to analyses of changes in PSA levels, pulmonary function changes comparing persons who develop ischemic heart disease and controls, and hearing threshold changes.

Publication of a new Bayseian method for statistically comparing several treatment groups to a control group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00015-34 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Baltimore Longitudinal Study of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Assoc. Scientific Director, BLSA	OSD, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	B. Hurley	IPA	UM
	C. Morrell	IPA	LC
	J.D. Pearson	Staff Fellow	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA

Other Investigators: See next page.

COOPERATING UNITS (if any)

Francis Scott Key Medical Center (FSKMC); National Institute of Dental Research (NIDR); University of Maryland, College Park (UM); Loyola College, Baltimore (LC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	.80	1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Baltimore Longitudinal Study of Aging (BLSA), the NIA's major research program on human aging, has been conducted at the Gerontology Research Center since 1958. The overall scientific goals of the BLSA are: 1) To identify differences among individuals of different ages and changes that occur in the serial observations of these individuals with the passage of time; to determine the relative contribution of aging, disease processes, cohort effects and secular effects in producing observed differences and changes; and to establish the degree of interrelation and/or interaction among these factors. 2) To expand scientific understanding about predictors and risk factors for specific diseases and for other end points related to successes and failures of adaptation to aging processes. The BLSA consists of a series of longitudinal and cross-sectional studies of varying degrees of interrelationships oriented toward description, prediction and intervention in human aging processes.

Scientists working with BLSA are assigned to 11 sections of 7 laboratories in addition to the LSB. The Chief, LSB, is the Associate Scientific Director, NIA for the BLSA and LSB staff administer and manage the BLSA as well as conduct research with it.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00624-02 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Baltimore Longitudinal Study of Aging (BLSA): Population Dynamics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Fozard	Chief & Assoc. Scientific Director	LSB, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
	C.S. Bacal	Physician Assistant	FSKMC
	C.L. Dent	Supervisory Biologist	LSB, NIA
	C.B. Willey	Program Assistant	LSB, NIA

COOPERATING UNITS *(if any)*

Francis Scott Key Medical Center

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	1.8	2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with optimal management and scientific description of the total BLSA population, which includes, as of 8/1/92, 1147 active participants (465 women; 682 men), 473 inactive (161 women; 312 men), and 538 deceased (43 women; 495 men). Active participants range in age from 20 to 97 years old.

Two major new research initiatives were approved and funded: the BLSA Vascular Initiative: Mechanisms of Race and Gender Differences in Hypertension with Aging, and the BLSA Perimenopausal Study. These projects, both of which include scientific questions about black-white differences, now drive recruitment of new BLSA participants. A new plan, adopted by the BLSA Steering Committee in March, 1992, calls for an additional 350 participants over the next 2 1/2 years, as follows: For the Vascular Initiative, 70 black females, 60 black males, 20 white females and 20 white males distributed over the age range. For the Perimenopausal Study, 100 black females, and 80 white, age 45 to 55. The BLSA has had health screening since 1989; however, additional health screening and the large black cohort required for the new initiatives present challenges for the development of new recruitment strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00625-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Baltimore Longitudinal Study of Aging (BLSA) Data Management

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. A. Shefrin	Computer Scientist	LSB,NIA
Others:			
	C. B. Eames	Programmer/Analyst	LSB,NIA
	N. S. Gittings	Programmer/Analyst	LSB,NIA
	G. S. Hammen	Computer Technician	LSB,NIA
	S. M. Pegram	Computer Technician	LSB,NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center (GRC), Longitudinal Studies Branch (LSB)

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	2.8	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Data Management work group is responsible for the storage of both paper and computer records generated by the BLSA. They perform the data entry of medical records and manage the data entry of many of the other data collected by the BLSA internal investigators and outside collaborators. Staff members manage the BLSA Computer System and its data base. They support both the administration of the BLSA as well as its scientific activities. Their functions include data extraction, processing, and analysis; consultation; training; hardware and software maintenance; and software development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00622-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health Disease Status in the BLSA: Clinical Health Evaluation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
	J.L. Fleg	Staff Cardiologist	LCS, NIA
	D. Kramer	Nurse Practitioner	FSKMC
	D. Binckley	Nurse Practitioner	FSKMC
	A. Rosenberg	Nurse Practitioner	FSKMC
	C. Kopac	Nurse Practitioner	FSKMC
	C. Bacal	Physician Assistant	FSKMC

COOPERATING UNITS *(if any)*

Francis Scott Key Medical Center (FSKMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.4	.4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new health questionnaire was implemented as of March 1, 1991 and continues to be used during the clinical health evaluation. It is a more thorough review of the participant's health status than was used prior to that date, but has markedly increased the time taken for the health evaluation, and for the time required to complete and sign out the charts. A revised physical examination protocol has been completed, but implementation has had to be delayed because of the new health questionnaire. Currently, we are developing an interval version of the health questionnaire to be used at visits subsequent to when the participant has completed the new health questionnaire. The interval questionnaire will probe changes in responses from one point in time to the next. Its goal is to identify the nature and degree that a symptom affects the life of the subject. A physical functioning inventory that will probe for mild to moderate disabilityhas been developed and is about to be implemented into the clinical evaluation. A research project was completed to examine the variability in blood pressure observed during the physical evaluation. The study resulted in standardizing when during the clinical evaluation the blood pressure is taken.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00626-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Age Changes in Visual Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	N.S. Gittings	Computer Programmer	LSB, NIA
	C.L. Dent	Testing Manager	LSB, NIA
	F. Schieber	Guest Researcher	USD
	D.W. Kline	Guest Researcher	UC
	T.S. Kline	Guest Researcher	UC

COOPERATING UNITS *(if any)*

University of South Dakota (USD)
University of Calgary (UC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.26	1.16	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Laboratory based assessment of contrast sensitivity for male and female participants of the BLSA was continued, increasing the sample size to over 500. The expanded contrast sensitivity dataset was reanalyzed and related to concomitant measures of visual problems reported by participants via a survey instrument. Intermediate and high spatial frequency contrast sensitivity declined slightly between ages 40-70 and more markedly thereafter. Contrast sensitivity measures were found to be related to self-reported visual difficulties encountered during the performance of routine daily activities as well as while driving an automobile.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00627-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Risk Factors for Age-Related Ocular Change

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sheila West	Associate Professor	Wilmer Institute JHU
		Guest Researcher	LSB NIA
Other:	Neil Bressler	Associate Professor	Wilmer Institute JHU
	Harry Quigley	Professor	Wilmer Institute JHU
	Evan Farmer	Professor, Dermatopathology	Wilmer Institute JHU
	Susan Vitale	Assistant Professor	Wilmer Institute JHU

COOPERATING UNITS *(if any)*

Wilmer Institute (Johns Hopkins University School of Medicine)
National Eye Institute

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine risk factors for the leading causes of blindness in the United States, age-related macular degeneration, cataracts, and glaucoma. Specifically, the study is examining the association of dermal elastotic degeneration and antioxidant vitamin status with age-related macular degeneration; the association of vitamin intake with cataract. A total of 719 participants age 40 and older with at least one visit prior to the ocular study were eligible, of whom 96% had macular and lens photographs to assess ocular status.

Low levels of serum vitamin E status were a risk factor for nuclear opacities. No other measures of antioxidant status, nor a combined antioxidant index, were associated with cataract.

No evidence of increased risks of either macular degeneration or high risk macular characteristics were found associated with elastotic degeneration. Females were significantly less likely to have high risk macular characteristics compared to males.

Low levels of serum vitamin E status were a risk factor for age-related maculopathy, as was low antioxidant status.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00628-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aging and Auditory Characteristics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandra Gordon-Salant	Guest Researcher	LSB, NIA
James L. Fozard	Chief	LSB, NIA
E. Jeffrey Metter	Medical Officer	LSB, NIA
Larry J. Brant	Mathematical Statistician	LSB, NIA

COOPERATING UNITS *(if any)*

University of Maryland

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.77	.27	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to combine assessment of hearing abilities among subjects of different ages over time, together with information from their communication and health histories. Medical and cognitive data collected from subjects in the longitudinal study will be examined with respect to the audiologic and case history data. The two principal objectives of this project are: A) To study the contribution of medical, genetic, dietary and social factors to age-related auditory dysfunction; and B) To determine to what extent age, independent of other etiologic factors, causes a deterioration in hearing abilities. During the past year, approximately 450 subjects from the BLSA have been tested on three new measures in the hearing protocol. These measures include assessment of pure-tone hearing sensitivity, sentence understanding in noise, and self-perceived hearing handicap. Of these participants, approximately 140 also were tested on the acoustic immittance series of electrophysiologic tests. We have continued to conduct a retrospective analysis of risk factors for apparent age-related hearing loss in men and women participants in the BLSA. Subjects included in this analysis exhibited significant deterioration in hearing during the course of data collection, but had no known cause for hearing impairment. Of the three modifiable risk factors examined, only blood pressure had a significant association with hearing loss.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00635-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Age Changes in Response Speed and Nerve Conduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Wood	Psychologist	LSB, NIA
	M. Vercruyssen	Consultant	UH

COOPERATING UNITS *(if any)*

University of Hawaii (UH)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.47	.17	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two measures of age-related slowing of behavior were analyzed to describe the age-related differences as well as age changes. The age-related changes in reaction time were not as robust as the cross-sectional differences suggesting that factors other than age are responsible for part of the age declines observed. Nerve conduction velocity decreased with older age with age difference becoming apparent around age fifty. Reciprocal manual movement speed declines relatively more rapidly with greater task difficulty in older age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG-00629-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health and Disease Status in the BLSA Men: Distribution of Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Staff Fellow	LSB, NIA
	G. Baker	Guest Researcher	LSB, NIA
	R. Kriner	Consultant	AARP

COOPERATING UNITS *(if any)*

American Association of Retired Persons (AARP)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00630-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health and Disease Status in the BLSA Women: Distribution of Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.J. Metter	Medical Officer	LSB, NIA
	D. Kramer	Nurse Practitioner	FSKMC
	D. Binckley	Nurse Practitioner	FSKMC
	A. Rosenberg	Nurse Practitioner	FSKMC
	C. Kopac	Nurse Practitioner	FSKMC
	C. Bacal	Physician Assistant	FSKMC
	L. Verbrugge	Guest Researcher	LSB

COOPERATING UNITS *(if any)*

Francis Scott Key Medical Center (FSKMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aging women experience life changes differently than men. Data from the BLSA are being analyzed to compare: 1) gender similarities and differences in demographics; 2) frequency of common diseases among men and women; 3) drug treatment of these common diseases with the focus being on hypertension and changes in treatment; 4) the differential effects on biological markers of natural versus artificially induced menopause; 5) the prevalence of urinary stress incontinence in women and its relationship to the aging process; and 6) effects of estrogen on skin elastosis. In the past year slow progress has been made in these studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00631-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health and Disease Status in the BLSA Men: Bias Issues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fleg	Sr. Staff Cardiologist	LCS, NIA
	J.L. Fozard	Chief	LSB, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Staff Fellow	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

THIS PROJECT HAS BEEN DISCONTINUED.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00632-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health and Disease Status in the BLSA Men: Perceived Health Status

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
	J.D. Pearson	Staff Fellow	LSB, NIA
	K. Elliott	Guest Investigator	GMU
	P. Knight	Guest Investigator	GMU
	D. Hiebert	Guest Investigator	GMU

COOPERATING UNITS *(if any)*

George Mason University (GMU)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is analyzing the association between health complaints as estimated by the Cornell Medical Index (CMI) and self perceived health. The project was originally begun to satisfy the requirements for a master's degree thesis that explored the responses in men over 55 years of age in a case-control study. Over the past year, analyses have been completed comparing men and women in the BLSA who have completed the CMI. The goal has been to explore what clinical symptoms and diseases contribute most to self-perceived health and what effect age has on this association. Both age and female sex resulted in increased numbers of positive responses on the CMI. A moderate degree of predictability was observed, with cardiovascular/hypertension questions being the best predictors of self perceived health for men at all ages, while for women musculoskeletal and gastrointestinal questions were predictive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00633-02 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health and Disease Status in the BLSA: The Prostate Gland

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E.J. Metter	Medical Officer	LSB, NIA
J.D. Pearson	Staff Fellow	LSB, NIA
J.L. Fozard	Chief	LSB, NIA
L.J. Brant	Statistician	LSB, NIA
R. Andres	Chief	LCP, NIA
S.M. Harman	Section Chief	LCP, NIA
H.A. Guess	Guest Researcher	MSD
H.B. Carter	Assistant Professor	JHU
P.C. Walsh	Professor	JHU

COOPERATING UNITS *(if any)*

Johns Hopkins University (JHU), Department of Urology
Merck, Sharp & Dome (MSD), Department of Epidemiology

LAB/BRANCH

Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, Gerontology Research Center

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the past year two studies have been completed. The first has examined prostate specific antigen as a marker of prostatic cancer. Longitudinal analyses demonstrated a significant age adjusted difference in rate of change in PSA between subjects with prostate cancer, BPH and controls. At a time when PSA did not differ between BPH and prostate cancer subjects (5 years before diagnosis), rate of change in PSA was significantly greater in subjects with prostate cancer. The findings suggest that rate of change in PSA may be a sensitive and specific early marker for the development of prostate cancer.

The second study estimated the PSA growth curves of BLSA participants with no evidence of prostate disease, BPH cases, and prostate cancer cases. Control participants exhibited minimal change in PSA thus suggesting that BPH is not a natural outcome of normal prostate growth, but is a separate pathogenic process. BPH cases showed a gradual and continuing acceleration in PSA with increasing age. Cancer cases exhibited an early slow linear phase of PSA growth followed by a rapid exponential growth phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00634-02 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Age Changes in Pulmonary Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Melvyn S. Tockman	Guest Researcher	LSB, NIA
Other:	Bernice H. Cohen	Guest Researcher	LSB, NIA
	Jerome L. Fleg	Sr. Staff Cardiologist	LCS, NIA
	James L. Fozard	Chief	LSB, NIA
	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jay Pearson	Staff Fellow	LSB, NIA

COOPERATING UNITS *(if any)*

The Johns Hopkins School of Hygiene
The Johns Hopkins School of Medicine

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.16	.31	.85

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Program in Pulmonary Aging at the GRC-BLSA has focused upon:

a. Development of a Mathematical Model of Pulmonary Aging

The age-related decline of individual pulmonary function (as a percent of BLSA predicted) may be described over longitudinal follow-up by a mixed-effects model which includes parameters for Intercept, Time Interval, (Time Interval)^2, and Age. A Gaussian distribution of the modeled pulmonary function provides age- and sex-specific percentile growth (decline) curves for normal pulmonary function as determined from BLSA healthy, nonsmokers, without evidence of heart disease.

b. Relation of Accelerated Decline in Pulmonary Function to Ischemic Heart Disease

A significant association has been observed between rate of pulmonary function decline and the development of IHD. After adjustment for risk factors of cardiovascular disease, a proportional hazards model describes a significant relative risk for incident IHD associated with pulmonary function decline. Using the mixed-effects model coefficients for FEV decline as the time-dependent covariate, along with covariates for FEV_1% $predicted_{Original}$, Smoking, Hypertension/cardiac medication, Cholesterol, and Participation status, a 7-fold relative risk for cardiac death and a 3-fold relative risk for hard cardiac endpoints was observed for BLSA participants in the worst quintiles of FEV decline.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00636-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Physical Activities in the BLSA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda P. Fried	Guest Researcher	LSB NIA
Other:	Jerome L. Fleg	Cardiologist	LCS NIA
	E. Gundy Zenger	Research Assistant	JHU
	Jordan D. Tobin	Chief, Applied Physiology	LCP NIA
	James L. Fozard	Chief	LSB NIA

COOPERATING UNITS *(if any)*

Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.03	0	.03

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study has analyzed data on physical activity pattern in participants in the Baltimore Longitudinal Study of Aging to determine how physical activity levels change as people get older, whether and how they differ between men and women, and whether regular physical activity has increased over the last 15 years. Answers to these questions will help to understand how best to evaluate physical activity levels in older persons compared to younger persons, and in women compared to men. The most valid approach is to determine the association of physical activity to health as people age. In addition, understanding of whether physical activity levels have increased over time is important in understanding the impact of public health campaigns in the U.S. This study has determined that physical activity levels decrease as people get older, and that older adults, both men and women, primarily obtain their physical activity from walking and housework. This information will help to change the questionnaires currently used for assessing physical activity. In the BLSA population, there has been a 21% increase in energy expenditure over a 15 year period. This significant increase indicates a major change in this important health habit. In addition, analyses suggest that the secular trend of increasing physical activity in this cohort is found at all ages of BLSA men, and alters the decline in physical activity expected with aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00639-01 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Individual Changes in Functioning with Age and Target Conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lois M. Verbrugge	Guest Researcher	LSB, NIA
	Ann L. Gruber-Baldini	Guest Researcher	LSB, NIA
Others:	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jay D. Pearson	Staff Fellow	LSB, NIA
	Larry Brant	Mathematical Statistician	LSB, NIA
	Cathy Dent	Supervisory Biologist	LSB, NIA
	James L. Fozard	Chief	LSB, NIA

COOPERATING UNITS *(if any)*

Institute of Gerontology, University of Michigan

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.6	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal research focus is to analyze longitudinal changes in functioning among BLSA subjects. The aims are to characterize intra-individual changes in 14 domains of activity and to study how these changes vary by sociodemographic and medical factors. The data for these analyses are derived from the "Activity Questionnaire II." It has been filled out by BLSA subjects at each visit since 1966. Subjects estimate the amount of time they spend on numerous specific activities, ranging from personal care to leisure. This data set is unique for its time stretch (up to 25 years for some subjects) and its content (the comprehensive scope of activities). Analyses on this data set include examinations of the cross-sectional, longitudinal, and secular patterns by age and gender. Cross-sectional analyses reveal consistent age and gender differences for participation in and time spent doing various activities, especially work, housework, childcare, and various discretionary activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00637-03 LSB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gender Differences and Individual Variability in Human Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Staff Fellow	LSB, NIA
	C.H. Morrell	IPA	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer, BLSA	LSB, NIA

COOPERATING UNITS *(if any)*

Department of Urology, Johns Hopkins University Medical School (HB Carter)
Laboratory of Cardiovascular Sciences, NIA (J.L. Fleg)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of gender differences and individual variability in age-related phenomena are being carried out to: 1) determine the "normal" range of variability in human aging, 2) identify potential sources of variability which may be responsive to intervention, and 3) determine if there are subgroups of individuals who are susceptible or resistant to various aspects of aging. The research combines the use of sophisticated statistical methodologies and the unique time depth and multidisciplinary breadth of the existing BLSA data base to examine issues related to the concepts of "normal" and "successful" aging, as well as to increase the power of traditional research designs. The statistical methods used include longitudinal regression models, time dependent proportional hazards analysis, and finite mixture models. Major findings include: 1) longitudinal rates of decline in hearing sensitivity are at least twice as fast in men compared to women, 2) prostate disease is the major determinant of the rates of change in prostate specific antigen, 3) longitudinal patterns of change in pulmonary function predict coronary events independent of other known cardiovascular risk factors. These findings represent significant contributions to the theoretical and methodological development of biomedical risk factor studies, as well as to an increased understanding of the dynamics of the aging process. Research is underway to develop more refined methods of studying variability in aging in order to develop theoretically and methodologically sound approaches to risk factor analysis which account for changes in an individual's covariates over time and the possibility that individuals differ in susceptibility or resistance to aging processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00638-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Health Promotion, Modifiable Risk Factors and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer, BLSA	LSB, NIA
	J.D. Pearson	Staff Fellow	LSB, NIA

COOPERATING UNITS *(if any)*

Health Services Research and Development Center, Johns Hopkins School of Hygiene and Public Health (P.S. German)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Unnecessary morbidity and mortality is an important problem which leads to increased health-care costs and can ultimately result in premature death. It has been estimated that approximately two thirds of mortality is due to potentially preventable causes - 1.2 million deaths (65%) and 8.4 million years of life lost before age 65 (63%). Principal factors associated with unnecessary morbidity and mortality include tobacco use, high blood pressure, improper nutrition, lack of screening and prevention services, alcohol abuse, and injury. This project uses longitudinal data from the Baltimore Longitudinal Study of Aging (BLSA) and other studies such as the Johns Hopkins' Nursing Home Study to examine the influence of modifiable risk factors on the occurrence of premature deaths and unnecessary morbidity and disability. Results from a study of newly admitted nursing home residents indicates that individuals with diagnoses of mental illness and other characteristics related to physical and mental function were found to be associated with greater use of physical restraints. These trends were true in nursing homes characterized by both high and low restraint use. Other predictors of the use of physical restraints include wandering, inability to dress, symptoms of depression, and the severity of cognitive impairment. Information from this study can have an impact on the training of nursing home staff to provide the best practices of managing nursing home residents' problems with the hope of improving the quality of care and living in a nursing home.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00623-04 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Statistical Methodology for the Analysis of Studies of Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
Others:	J.D. Pearson	Staff Fellow	LSB, NIA
	C.H. Morrell	IPA	LSB, NIA

COOPERATING UNITS *(if any)*

Department of Mathematical Sciences, Loyola College in Maryland (C.H. Morrell)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The applied and theoretical development of statistical methodology is progressing in the areas of biological and epidemiological models, mixture models for describing age changes in distributions of biological markers of morbidity and mortality, multiple comparisons, survival analysis, and the design of experiments, each of which is applicable to longitudinal studies and other studies of aging. The research utilizes various types of statistical models - regression models for studying risk factors' association with outcomes observed in prospective studies, and mixed-effects models for longitudinal data which consider both within- and between-subject variation in analyzing the repeated measurements for all individuals in the study population. Other techniques used include Bayesian, maximum likelihood and numerical computing methods. The methodology created provides original contributions to experimental testing associated with longitudinal studies, simultaneous comparison of various specified experimental effects, epidemiological study of disease states, survival or failure analyses of longitudinal observations representing growth, physical and mental disability, and other biological and behavioral changes in humans and animals. A major emphasis of this research project is on the development of methods which yield cogent yet easily understood results when applied to data. Mixed-effects regression models have been completed for two different aging studies. One study of nursing home patients resulted in the first comprehensive publication of such models in the aging literature. The second study of longitudinal changes in blood pressure found that individuals with rates of change in systolic blood pressure greater and smaller than the average rates of change had an increased prevalence of coronary heart disease.

ANNUAL REPORT OF THE LABORATORY OF BEHAVIORAL SCIENCES

NATIONAL INSTITUTE ON AGING

Significant Administrative Events

There have been a number of personnel changes during FY-1991: Dr. J. A. Haythornthwaite, who was a Senior Staff Fellow in the Behavioral Medicine Section (BMS), accepted the position of Director, Behavioral Medicine Clinic at The Johns Hopkins University School of Medicine. Dr. A. Bagrov, Director, Coronary Care Unit of the I. I. Dzhanelidze Institute of Medical Research (St. Petersburg, Russia) has accepted a position in BMS as a Visiting Associate. Dr. M. Lipp, (Universidade Catolica de Campinas, Rio de Janiero) completed a one-year term as a Guest Researcher in BMS. Dr. R. Kawate, (Tokyo University, Japan) completed a two-year term as a Visiting Fellow in the Behavioral Physiology Section (BPS). Dr. M. Kawate (Tokyo University, Japan) completed a two-year term as Guest Researcher in BPS. Mr. O. Schechtman (Petach-Tikvah, Israel, and the University of Florida) accepted a position as Visiting Associate in BPS.

LBS is developing an intramural research program in Behavioral Nursing. This program derives from the facts that: (1) approximately 30% of all persons 65 years or older are functionally disabled to some degree; (2) almost all of these persons suffer from incurable illnesses; (3) the primary health care providers for these people are nurses; and (4) the primary clinical goal is to optimize self-sufficiency for these people. Thus, the principal interventions require an integration of behavioral and medical treatment strategies which can be implemented by patients and caregivers under the supervision of home, health-care nurses, and nursing home, nursing staffs. Dr. Ann Myers has accepted a position as a Senior Staff Fellow, and will be developing a research program to study mobility in community-dwelling elderly and nursing-home residents. She will be joined by Dr. Mary Palmer, a Senior Staff Fellow in the National Center for Nursing Research (NCNR, NIH), who is detailed to LBS. Dr. Palmer, has just completed a two-year IRTA fellowship in LBS where she studied the effectiveness of behavioral treatment of incontinence in nursing-home residents. She will continue her work on continence care, and also will collaborate with Dr. Myers on her work on mobility in nursing-home residents.

Research Advances

Nocturnal Hemodynamics

LBS is carrying out a research program on the physiological and possible clinical aspects of overnight changes in the circulation. This program includes studies of animal models as well as patients; the latter usually based on findings first made in the animal laboratory. We have described a highly-reproducible, hemodynamic

pattern in monkeys that is characterized by an overnight fall in cardiac output, associated primarily with a fall in heart rate since stroke volume normally changes little overnight; an overnight fall in central venous pressure; and, a rise in total peripheral resistance. We hypothesized that this pattern was mediated, largely, by a fall in plasma volume since monkeys, like people, lose fluid volume overnight, which they do not replace. This hypothesis is supported by several other observations: (1) last year, we showed that the hematocrit is consistently higher in the morning than it is in the evening; (2) this year, we found that the viscosity of whole blood is higher in the morning than in the evening; (3) Echo-Doppler cardiographic studies completed this year have shown that when heart rate is kept constant (by atrial pacing), end-diastolic volume and stroke volume are lower in the morning than in the preceding evening. The echocardiographic studies also showed for the first time that the systolic functions of the heart (rate- adjusted, pre-ejection period, and ejection fraction) are weaker in the morning, and cardiac contractility (normalized systolic ejection rate) is also poorer in the morning. Another study has shown that the nocturnal hemodynamic pattern described above occurs whether the animals are allowed to change posture or are maintained in the same posture overnight. This last finding, coupled with earlier studies that showed this pattern was present even if the sympathetic nervous system was blocked, or if the conversion of angiotensin I to angiotensin II was prevented, shows clearly that these overnight changes in hemodynamic functions are very stable phenomena. One other finding from the nonhuman primate laboratory is that there is a decline in echo-Doppler cardiographic indices of cardiac function if heart rate is not allowed to fall overnight. This finding is consistent with an earlier finding that if one does not allow the heart rate to fall overnight, there is a significant decline in hemodynamic signs of cardiac function.

These studies in laboratory animals have generated a number of studies in patients. One such study, which is nearing completion, is examining echocardiographically-measured cardiac performance in patients with implanted pacemakers: this study compares cardiac function in the morning and evening, after three weeks of pacing at a high, normal rate, to diurnal function after three weeks of pacing at a low, normal rate. Preliminary findings indicate that cardiac function is poorer in the morning than in the evening, and poorer after three weeks of pacing at the higher rate relative to the lower rate. These findings suggest that a pacemaker rate that is set high enough to support normal daytime activities may be overdriving the heart at night. A second study, which has just begun, will compare overnight changes in hematocrit, whole blood and plasma viscosity in patients with ischemic heart disease to normal controls. A third study is planned, which is designed to examine diurnal changes in hemodynamic function in wheelchair-bound patients to age-matched controls to learn if there are any hemodynamic consequences of the long-term postural effects caused by sitting in a wheelchair.

Thermoregulation

Studies completed this year have shown, by direct measurement, that sympathetic nervous activity to brown adipose tissue (BAT) is greater in cold-acclimated mice (animals exposed to cold for 3 hours every other week for six weeks) than in mice not so trained. Not only is the resting level of nervous activity greater in the acclimated mice, but also their nervous response to a cold challenge is greater. Furthermore, the acclimated animals maintain core temperature longer during cold challenge than do the unacclimated mice. These findings indicate either that the ability of acclimated mice to detect cold is greater, or that their set point for sympathetic drive to brown adipose tissue is set higher. A second study has shown that the sympathetic nervous activity to brown adipose tissue in noncold-adapted, aged mice is comparable to that of cold-adapted, adult animals. This indicates that old animals are capable of sensing cold and of emitting appropriate efferent nervous responses to it, but they are less effective at responding to cold since their ability to maintain core temperature is poorer than is that of adult mice. Thus, older animals are either less able to produce heat, or they are less able to conserve heat. A third study in this program showed that old mice were less able to maintain body temperature under anesthesia than were adult animals because anesthesia suppressed metabolic heat production more in the aged animals.

Blood Pressure Regulation

Previous research in laboratory animals showed that behavioral conditioning schedules, which generate sustained hypopnea, peripheral vasoconstriction, and bradycardia, potentiate the development of hypertension when the animals also are on a high-sodium intake: neither the conditioning schedules nor the high-sodium intake alone are sufficient to cause hypertension. Recent studies in BMS have shown that normal human volunteers can be trained to sustain a low frequency/normal tidal volume, breathing pattern, which maintains pCO_2 at the upper-end of the normal range. This breathing pattern is associated with higher blood pressure, lower heart rate and no change in stroke volume, indicating that the elevated blood pressure is mediated by a rise in total peripheral resistance. Other studies have shown that neither directly measured, sympathetic nervous activity in skeletal muscle, nor sympathetic nervous activity to the heart (estimated from beat-to-beat histograms) is increased during low-frequency breathing. These findings support the hypothesis that the rise in pressure is not mediated by sympathetic nervous effects. Another study completed this year has shown that mild, hypercapnic breathing is associated with a decrease in blood pH and a delayed rise in bicarbonate ions. These last findings are consistent with the hypothesis that hypercapnic breathing could increase peripheral resistance via a sodium-hydrogen exchange mechanism, and that behaviorally mediated effects could potentiate sustained rises in blood pressure in sodium sensitive subjects via this mechanism rather than through sustained arousal of the sympathetic nervous system.

This last hypothesis gains further support from previous studies in our laboratory: we found that most people have several periods of sustained, mild hypoventilation throughout the day, and that these are most likely to occur in social situations in which the subject is interacting with another person.

The role of social behaviors in mediating "inhibitory breathing" is being explored in the laboratory as well as in field studies. A study that is now nearing completion examines the interaction between cardiovascular and respiratory responses in normotensive and hypertensive subjects during role playing. Subjects are presented with scenes in which they are required to respond extemporaneously, but assertively to challenges. The choice of these situations is based on earlier findings that suggest that hypertensive subjects may have difficulty dealing appropriately with such challenges, and that they may respond with a behavioral pattern that includes inhibitory breathing. Although this study is still in progress, the results to date indicate that both patients and controls respond to the challenge with significant pressor responses and minimal heart rate changes. In addition, the hypertensive subjects have elevated levels of pCO_2 at baseline, and respond to the social challenges with exacerbated responses. This study also includes a relaxation therapy component. It will be interesting to see if relaxation attenuates the pressor and hypercapnic reactivity of the patients.

Continence Care in the Nursing Home

Incontinence is extremely common among nursing home residents. Although there are a number of factors that contribute to the cause of incontinence in this setting, it is clear that a major factor in its treatment is improved nursing care. A study completed this year showed that a prompted voiding intervention could be implemented and managed by the indigenous, nursing staff in a community-based nursing home. Once the staff had been trained to implement and supervise the prompted voiding treatment, they were able to increase dryness in the study patients from 45% to 64%, and to maintain this level over the 12-week study period. Thus, these findings replicated and extended previous results from this laboratory. Earlier findings had shown that a research staff in a hospital-based nursing home were able to implement the treatment and maintain its beneficial effects; the present findings show that indigenous staff in a community-based home can be equally effective. Thus, this research has provided a model for the implementation of current federal guidelines for continence care in the nursing homes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00063-24 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Learned Modification of Visceral Functions in Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Bernard T. Engel, Chief	LBS, NIA
OTHERS:	Mark I. Talan, Medical Officer (Research)	LBS, NIA
	Paul H. Chew, IPA	LBS, NIA

COOPERATING UNITS (if any)

Johns Hopkins Univ. Medical School, Francis Scott Key Medical Center, Division of Cardiology, Baltimore, MD (P. Chew)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.75	3.20	2.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the role of the central nervous system in the regulation of the circulation. Instrumental conditioning is used to directly modify cardiovascular responses. Naturally occurring, adaptive responses are examined by monitoring overnight changes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00072-07 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Assessment and Treatment of Incontinence in Nursing Home Residents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: Bernard T. Engel, Chief LBS, NIA

OTHERS: Mary H. Palmer, Senior Staff Fellow NCNR, NIH

COOPERATING UNITS (if any)

Univ. Pittsburgh School of Medicine, Pittsburgh, PA (L. Burgio and K. Burgio): Francis Scott Key Medical Center, Johns Hopkins Univ. School of Medicine (J. Marks); Lorien Nursing Home, Baltimore, MD (R. Bennett)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.15	0.60

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Incontinence is a major reason for institutionalizing elderly persons and is common in nursing homes. This project is designed to evaluate the effectiveness of behavioral treatment techniques as well as staff management techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00073-04 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology of Thermoregulation and Aging in Rodents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Mark I. Talan, Medical Officer (Research)	LBS, NIA
OTHERS:	Bernard T. Engel, Chief	LBS, NIA
	Ryoichi Kawate, Visiting Fellow	LBS, NIA
	Mariko Kawate, Guest Researcher	LBS, NIA
	Orit Schechtman, Visiting Associate	LBS, NIA

COOPERATING UNITS (if any)

Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (A. Sato, Y. Sato)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.30	3.55	2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is: (1) to investigate age-related changes in thermoregulation; and, (2) to examine the physiological mechanisms underlying these changes.

We have demonstrated that aged mice have diminished cold tolerance and are not able to adapt to repeated cold exposure. The cause of these age-related aberrations in thermoregulation appears to be, in part, a reduction in metabolic heat production due to change in brown adipose issue (BAT) and, in part, a reduction in heat conservation.

Efferent sympathetic nervous responses to BAT are enhanced in both cold-acclimated and aged animals, which suggests that the sympathetic nervous system plays a major role in cold acclimation, but is not responsible for the aged-related decline in thermoregulation. Our results show that sympathetic nervous activity increases with age to adapt to age-related declines in effector organ function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00074-02 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Factors in Transient Myocardial Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Jennifer A. Haythornthwaite, Senior Staff Fellow LBS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Section, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00600-04 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Respiratory Factors in Blood Pressure Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David E. Anderson, Chief, Behavioral Physiology Section LBS, NIA

OTHERS: Paul T. Chew, IPA LBS, NIA

COOPERATING UNITS (if any)

Johns Hopkins Univ. Medical School, Francis Scott Key Medical Center, Division of Cardiology, Baltimore, MD (P. Chew)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	0.9	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies showed that aversive conditioning procedures which elicited sustained hypoventilatory breathing and peripheral vasoconstriction potentiated the development of experimental hypertension in laboratory animals, but only under conditions of high sodium intake. This hypoventilatory breathing pattern has been observed to occur episodically in humans in their natural environments, to occur more frequently at work and in social situations, and to be accompanied by acute increases in blood pressure, but not heart rate. During the past year, humans have been trained to comfortably maintain hypoventilation-induced hypercapnia in the laboratory using a feedback procedure. The breathing pattern has been associated with small but sustained increases in blood pressure, decreases in heart rate, increases in pCO_2 and decreases in pH. Bicarbonate ion concentrations showed a delayed rise under these conditions. There was no evidence of increased sympathetic nervous system activity in the heart. These findings are consistent with the hypothesis that sustained mild hypercapnic breathing, itself, could potentiate the development of hypertension in sodium-loaded individuals via activation of a cell membrane sodium-hydrogen exchanger, expansion of plasma volume and stimulation of circulating sodium pump inhibiting compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00603-03 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Implications of Nocturnal Hemodynamic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Paul H. Chew, IPA	LBS, NIA
OTHERS:	Bernard T. Engel, Chief	LBS, NIA
	Mark I. Talan, Medical Officer (Research)	LBS, NIA

COOPERATING UNITS (if any)

Johns Hopkins Univ. Medical School, Francis Scott Key Medical Center, Division of Cardiology, Baltimore, MD 21224 (P. Chew)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.85	0.65	0.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in animals have shown significant nocturnal hemodynamic patterns that may contribute to morbid changes in cardiovascular functions in patients with heart diseases. This project is designed to evaluate overnight cardiovascular effects in select patient groups and compare these to control groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00604-03

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Factors in Blood Pressure Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Jennifer A. Haythornthwaite, Sr. Staff Fellow LBS, NIA

Marilda N. Lipp, Guest Researcher LBS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	2.0	1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies with laboratory animals have shown that, although neither aversive conditioning procedures alone nor salt loading alone increased 24-hr mean blood pressure, a combination of aversive conditioning and high sodium intake were sufficient to engender experimental hypertension within days. The present project extends these findings to behavioral analysis of the regulation of human blood pressure.

Two studies have now shown that 14 days of high sodium intake by medical students preparing for examinations increased resting blood pressure (but not heart rate), while neither preparation for examinations in a context of normal sodium intake, nor sodium loading during summer vacation, had any significant effect. Physiological changes accompanying this model of human experimental hypertension have been characterized. Since the development of primary hypertension may involve deficits in assertive behavior, a study has been initiated to assess cardiorespiratory responses of normotensive and borderline hypertensive humans to laboratory situations of simulated social stress. This study also investigates effects of training in relaxation on social stress reactivity and on ambulatory blood pressure. Preliminary data indicate that hypertensive subjects show higher levels of pCO_2 than normotensive subjects under laboratory conditions. This response, if habitual, could mediate increased blood pressure sensitivity to high sodium intake.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00605-01 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cardiovascular Interactions of Stress and Salt in the Micropig

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Michael Crowell, Guest Researcher LBS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00606-02 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Intervention for Alzheimer's Patients and Their Caregivers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Bernard T. Engel, Chief	LBS, NIA
OTHERS:	Maria Mannarino, Medical Officer	OEA, NIA
	Carol Fuchs-Kinslow, Clinical Social Worker	LNS, NIA
	Marie Holly, Clinical Nurse	LNS, NIA

COOPERATING UNITS (if any)

Office of Extramural Affairs, NIA (M. Mannarino); Laboratory of Neurosciences, NIA(C. Fuchs-Kinslow; M. Holly)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.80	0.30	0.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to evaluate the efficacy of relaxation training as a treatment for the distress of the caregivers of Alzheimer's patients, and for the patients themselves. The treatment is specifically designed to enable the caregiver to cope more effectively with her/his distress, and to provide the caregivers with an effective means of managing many of the disruptive behaviors of the patient.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00607-01 LBS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Effects on Blood Pressure and Circulating Sodium-Pump Inhibitors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Alexey Y. Bagrov, Visiting Associate LBS, NIA
Edward G. Lakatta, Chief, Laboratory of Cardiovascular Science LCS, NIA

COOPERATING UNITS (if any)

University of Maryland School of Medicine, Baltimore, MD (J. Hamlyn)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.30	0.20	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Epidemiological studies have shown that mean blood pressure and the prevalence of hypertension both increase with age. A possible role for dietary sodium intake in the blood pressure increase in aging individuals has been documented, but little information is available concerning possible age-associated changes in circulating hormones that affect sodium transport across vascular smooth muscle. The present study assesses individual differences in levels of two circulating sodium-pump inhibiting hormones as predictors of age-associated changes in resting blood pressure. Morning supine blood pressure and endogenous ouabain and digitalis-like factor will be measured in participants in the Baltimore Longitudinal Study on Aging meeting the inclusion criteria. The study also investigates possible differences in circulating sodium-pump inhibitors as a function of race and gender. Data supporting the hypothesis of a positive correlation between resting blood pressure and circulating sodium-pump inhibitors could lead to pharmacological and nonpharmacological intervention in hypertension focusing on either or both of these sodium-pump inhibitors and their determinants.

ANNUAL REPORT OF THE LABORATORY OF BIOLOGICAL CHEMISTRY

NATIONAL INSTITUTE ON AGING

The Laboratory is continuing to develop strong research programs relevant to basic mechanisms of aging and age associated diseases and disabilities. These efforts are divided between two groups. The Molecular Neurobiology Unit and the Cell Biology Unit. The Molecular Neurobiology Unit had a significant set back in studies on a transgenic mouse overexpressing a portion of the amyloid precursor protein. This was a collaboration project with a university group who prepared the transgenic mice. While the original studies, conducted on a limited number of animals, suggested that the transgenic animals had pathological changes in the brain that resembled those in Alzheimer's Disease, studies on additional animals did not confirm these results and they were retracted. Transgenic mice still represent the most likely way to develop animal models of Alzheimer's Disease. However, progress was made in other areas including finding a deficiency of brain-derived neurotrophic factor in affected areas of brain in Alzheimer's Disease. Also, an unusual splice form of the NMDA receptor has been detected and mapped in the brain.

The Cell Biology Unit has four strong programs including aging and cancer, the occurrence of deletions of mitochondrial DNA with age, bone induction and cartilage. Progress has been made in developing animal models of spontaneous breast cancer and human prostate cancer. Also, a simple quantitative convenient in vivo angeogenesis assay has been developed. Aging accelerates changes in mitochondrial DNA, cartilage and bone formation. The underlying causes are being elucidated and attempts are being made to reverse or arrest these changes. New approaches to correct age-associated disabilities range from gene therapy, to growth factors, to newly developed antioxidants that target sensitive processes.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00053 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Renal Failure in Age-Associated Changes in Wistar Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: C.R. Filburn Research Chemist, LBC GRC NIA

Others:

L. Cheng Research Chemist LCS GRC NIA

COOPERATING UNITS (if any)

S.G. Massry, G.Z. Fada, X.-J. Zhou Div. of Nephrol., Dept. of Med. Univ.So.Calif.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00055-03 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Age on Osteogenic Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: C.Tony Liang Research Chemist, LBC GRC NIA

Others:

Janice Barnes	Biologist	LBC GRC NIA
Sonia Williams	Staff Fellow	LBC GRC NIA
Rodolfo Quarto	Visiting Associate	LBC GRC NIA
Hiroshi Tanaka	Visiting Associate	LBC GRC NIA

COOPERATING UNITS (if any)

Dr. Gideon Rodan, Dir., Depart. of Bone Biology and Osteoporosis Res., Merck, Sharp and Dohme Res. Lab.

Dr. Mark Bolander, Orthopedic Surgery, Mayo Clinic

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

o (a) Human subjects o (b) Human tissues X (c) Neither

o (a1) Minors

o (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main goal of this study is to establish an animal model system which can be used to monitor age changes in bone activity which may relate to defects underlying osteoporosis and allow novel therapies to be tested. An injury model, involving bone marrow aspiration was used to induce rapid production of bone in the marrow cavity. This process can be quantitated by measuring the expression of genes involved in bone formation and resorption. This model was also used to examine the response of aged animals and indicated that bone induction was severly attenuated with age. Currently, we are using this model to evaluate the effectiveness of various novel interventions including growth factors, stem cells and gene therapy in restoring the ability of old animals to form bones.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00056-03 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Mitochondria-Associated Tumor Hexokinase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: C.R. Filburn Research Chemist, LBC GRC NIA

COOPERATING UNITS (if any)

P.L. Pedersen, Lab. Molecular Bioenergetics, Dept.Biol.Chem., Johns Hopkins Univ.
K.A. Arora, Lab. Molecular Bioenergetics, Dept.Biol.Chem., Johns Hopkins Univ.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A mitochondrial-binding hexokinase found in brain and in hepatomas is believed to be very important in regulation of energy metabolism. This enzyme is 100 kDa with two 50 kDa, highly homologous halves. The N-terminal half is believed to contain a glucose-binding site important in product inhibition and binding to the mitochondrial outer membrane. Mutagenesis of specific amino acids in the N-terminal half fail to affect activity a product inhibition. Further studies are needed on this hexokinase to define the sites involved in product inhibition and mitochondrial binding. Since this hexokinase plays a major role in neuronal energy metabolism, defining its regulation is of fundamental importance to understanding aberrations in energy metabolism that may underly age-associated diseases.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00057-03 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Matrix Gene Expression in Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: Walter E. Horton, Jr. Senior Staff Fellow, LBC GRC NIA

Others:

Richard Balakir	Chemist	LBC GRC NIA
Patricia Precht	Biologist	LBC GRC NIA
Douglass Bradham	Staff Fellow	LBC GRC NIA
Liqun Wang	Visiting Fellow	LBC GRC NIA
Darryl Murray	Biologist	LBC GRC NIA
Beatrix InderWiesche	SIS	LBC GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cartilage is a unique tissue that functions to cushion the impact between bones and is sensitive to age-associated changes that ultimately leads to osteoarthritis, a disease afflicting millions of elderly individuals. Collagen II is an abundant component of the cartilage matrix and its expression by the chondrocyte is altered during the progression of osteoarthritis. We are studying the molecular mechanisms that regulate chondrocyte gene expression with an emphasis on the control of type II collagen synthesis. The collagen II gene contains a DNA sequence that functions as a chondroctye-specific enhancer of transcripton and cell specific expression. Using DNA-affinity chromatography, we have partially purified a group of 3-4 proteins that bind to a specific DNA sequence in the enhancer and we are currently attempting to microsequence these proteins. Also, we have obtained a cDNA that encodes a protein that binds to the recognition sequence and we are currently characterizing this clone further. We are also characterizing **in vitro** and **in vivo** models to study the regulation of chondrocyte matrix synthesis and proliferation by growth factors and the activation of chondrogenesis by drugs. Finally, we have initiated studies to determine the pattern of gene expression in articular cartilage from different aged rats. These studies should eventually allow us to design novel therapies for osteoarthritis by stimulating chondrocyte proliferation and matrix synthesis.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00058-03 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor growth and metastasis: aging and cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: Antonino Passaniti Senior Staff Fellow, LBC GRC NIA

Others:

Joseph Haney	Biologist	LBC GRC NIA
Yue Guo	Visiting Fellow	LBC GRC NIA
Roberto Pili	Visiting Fellow	LBC GRC NIA
Teresa Novick	SIS	LBC GRC NIA
Hayao Nakanishi	Guest Researcher	LBC GRC NIA

COOPERATING UNITS (if any)

John Isaacs, Professor of Oncology, Johns Hopkins Univ.
Hynda Kleinman, Chief, Cell Biology Section, LDB, NIDR
Robert M. Taylor, Professor of Pathology, Johns Hopkins Univ., FSK
Marc Lippman, Director, Lombardi Cancer Center, Georgetown Univ.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.25	4.0	1.25

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed several models to investigate the changes in cancer incidence and growth with age. An animal model using nude mice to grow human prostate, and small cell lung carcinoma cells employs extracellular matrix to stimulate tumor growth allowing anti-tumor agents to be tested. The same matrix proteins were shown to also enhance tumor growth in older animals perhaps by overcoming host immune defense. A quantitative angiogenesis assay was developed to measure the role of neovascularization in tumor growth and atherosclerosis. Using this assay, inhibitors and promoters of angiogenesis were identified. Vascular smooth muscle cells inhibit the angiogenic response suggests that they secrete inhibitors. We have also developed a rat model for spontaneous, mammary neoplasms by isolating cells from spontaneous breast tumors of aged rats which will be used to assess genetic alterations associated with aging and tumorigenesis.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00059-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Occurrence of Deletions of Mitochondrial DNA in Tissues of the GRC Wistar Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: C. R. Filburn Research Chemist, LBC GRC NIA

Others:

W. Edris	Biologist	LBC GRC NIA
M. Tamatani	Visiting Fellow	LBC GRC NIA
R. Alexander	Laboratory Aide	LBC GRC NIA
R. Cutler	Volunteer	LBC GRC NIA

COOPERATING UNITS (if any)

C. Stine, Assistant Professor, Johns Hopkins Univ., School of Medicine
R. Hansford, Senior Investigator, LCS, GRC, NIA

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Damage to mitochondrial DNA has for years been proposed to play a major role in aging process. Recent studies have shown that mutations are in fact involved in some genetic diseases characterized by muscle weakness and encephalopathy. In an effort to develop an animal model to study the causes and consequences of mitochondrial DNA damage, the Wistar rat was examined to assess the presence and/or level of deletions. A 4.8kb deletion was found and shown to increase markedly with age in rat liver. This deletion corresponds very closely to a 5.0kb deletion commonly found in humans with mitochondrial myopathies and known to increase with age. Mouse and monkey mitochondrial DNA are also being studied in hopes of developing additional models. This form of mitochondrial DNA damage may be important in muscle weakness and/or atrophy and neuronal degeneration that occurs with age.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00500-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transgenic and Cellular Models of Alzheimer's Neuropathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: Gerald A. Higgins Research Biologist, LBC GRC NIA

Others:

Jeffrey Chernak	Senior Staff Fellow	LBC GRC NIA
Boyu Zhao	Visiting Fellow	LBC GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brain from persons with Alzheimer's disease contains plague-like deposits of a peptide derived from the Amyloid Precursor Protein (APP). The function of this protein is not known but deposits appear to be related to the development of the disease. These studies are intended to delineate the function of APP and its role in Alzheimer's disease.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00501-02 LBC

PERIOD COVERED

October 1, 1990 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alternative Splicing of the Amyloid Precursor Protein Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

PI: Gerald A. Higgins Research Biologist, MNU LBC GRC NIA

Others:

Lisa Kale	Biologist	MNU LBC GRC NIA
Cheryl A. Sherman	Biologist	MNU LBC GRC NIA

COOPERATING UNITS (if any)

Karen Beeman, Dept. of Biology, Johns Hopkins Univ.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major component of the plaques and tangles found in Alzheimer's disease is the ßA/4 protein which is derived from the amyloid precursor protein (APP) by proteolytic cleavage. The specific events leading to this deposition are presently unknown but different isoforms of APP may play a role in this phenomenon. A feature that distinguishes APP isoforms is the presence or absence of a Kunitz-type serine protease inhibitor (KPI) domain in the extracellular amino terminal portion of the molecule. One APP isoform lacking the KPI domain, is a 695 amino acid protein (APP-695) largely restricted in its expression to the central nervous system. At the mRNA level, the APP gene produces multiple transcripts in the human and rodent central nervous system. The most abundant species of APP mRNA expressed in the CNS of rodents and other lower mammals lacks sequences encoding the KPI domain, while KPI-containing forms of APP message, such as APP-751, are expressed at relatively higher levels in the human brain. Our work shows that the ubiquitous, KPI-containing forms of APP mRNA appear to accumulate during aging of the human and rat CNS, and increases in the APP-751/APP-695 ratio are associated with Alzheimer's disease in affected bain regions. In addition, switching of APP RNA splicing to APP-695 in cognitively impaired, aged rats can be regulated by exogenous administration of nerve growth factor (NGF), which also reverses spatial memory impairments associated with aging in these rats. The results suggest that abnormal expression of different isoforms of the APP protein may be important in the etiology of Alzheimer's disease.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00502 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Tau Promoter Sequences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Gerald A. Higgins Research Biologist, LBC GRC NIA

Others:

William Brown Visiting Fellow LBC GRC NIA

COOPERATING UNITS (if any)

Kenneth Kosik, Harvard Medical School

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00503-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Neurotrophin Expression in Alzheimer's Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Gerald A. Higgins Research Biologist, MNU LBC GRC NIA

Others:

Carey Byrne	Biologist	MNU LBC GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects X (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Certain proteins, referred to as growth factors, sustain neural cells in the brain. Examination of the expression of such factors with age and disease may reveal factors associated with cell death, as well as define new therapeutic approaches for Alzheimer's disease. Polypeptides such as neuronal growth factors or myelin-associated neuronal regeneration inhibitory factors may be involved in neuronal survivial in degenerative diseases. Initial studies have focused on regulation of the neurotrophin class of nerve growth factor (NGF-like) and their receptors (the trk family of proto-oncogenes) in Alzheimer's disease, while future studies will be directed towards investigation of newly characterized polypeptides that are associated with inhibition of neuronal regeneration in the CNS.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00504-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth Factor Activity in Neurodegeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Smita Kittur Senior Staff Fellow, MNU LBC GRC NIA

Others:

Hidetoshi Endo Visiting Fellow MNU LBC GRC NIA

COOPERATING UNITS (if any)

Dr. William Marksbery, M.D., Prof., Department of Neurology, University of Kentucky

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects X (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies made on brain fluids and tissues may assist in developing new diagnostic and therapeutic procedures for Alzheimer's disease (AD). Patients with AD suffer mental deterioration associated with neurofibrillary tangle and senile plaque formation. The excessive sprouting seen in the brains of AD patients may be due to a growth factor activity. To test this hypothesis, we treated neuronal cells in culture with brain extracts from AD patients and normal controls. The results show excessive branching activity of neuronal cells in culture, treated with AD brain extract suggesting overproduction of growth factor activity in AD brains. To find out if the growth factor laminin was increased in AD patients, we measured laminin in CSF and serum of AD patients and showed no difference in laminin values between AD patients and age matched controls but showed an age associated increase in laminin.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00505-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitatory AA Receptors' Role in Alzheimer's & Other Neurodegenerative Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: John W. Kusiak Research Chemist, MNU LBC GRC NIA

Others:

Darrell D. Norton	Biologist	MNU LBC GRC NIA
John A. Izzo	IRTA	MNU LBC GRC NIA
Cheryl A. Sherman	Biologist	MNU LBC GRC NIA
Guang Bai	Senior Staff Fellow	MNU LBC GRC NIA
Laura Gerald	Summer Aide	MNU LBC GRC NIA

COOPERATING UNITS (if any)

Molecular Physiology & Genetics Section, LCMB, GRC, NIA

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	3.0	.25

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The receptors for certain amino acids have complex roles in the brain in learning, memory and even in inducing neuronal death. These receptors are being studied for their possible role in various age-associated diseases including Alzheimer's disease. The major emphasis of this project is the investigation of the role of excitatory amino acids (EAA) receptors in neurodegenerative disorders of aging. This year we discovered a splice variant form of the NMDA subtype of EAA receptor. This isoform contains a deletion of 35 amino acids in the carboxy tail of the molecule. The deleted amino acids were found to be encoded on a separate exon in the rat genome. The deletion form is predominantly expressed in the cerebellum and brain stem while the full length form is found in rostral regions of the brain. We have found that both undifferentiated and differentiated PC-12 cells express both flip and flop forms of the GLUR-2 submit of the AMPA receptor, and that some previously uncharacterized splicing of this gene may occur in cell culture. We have just initiated work to clone and characterize the promoter region of the NMDA receptor. This work is critical in determining how the expression of the NMDA receptor is regulated at the molecular level. Finally, neural cell culture is being explored to examine NMDA and AMPA receptor subunit changes under conditions mimicking neurotoxicity and neuroprotection.

ANNUAL REPORT OF THE LABORATORY OF CARDIOVASCULAR SCIENCE
NATIONAL INSTITUTE ON AGING

The overall goals of the Laboratory of Cardiovascular Science are (1) to identify age-related changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to study myocardial structure and function gene expression, and response to pharmacologic therapeutics in disease models, and to determine how age interacts with these chronically altered cardiac states to determine the level of myocardial function; (3) to study basic mechanisms in excitation-contraction coupling and of energy-yielding oxidative pathways in cardiac muscle; (4) to determine the chemical nature and sequence of intermediate reactions controlling the movement of ions through ionic channels and pumps present in myocardium, specifically with respect to how the affinity, capacity and selectivity of ion translocation through membranes are affected by aging and disease; and (5) to determine mechanisms that govern normal and abnormal function of vascular and endothelial cells. In meeting these objectives, studies are performed in human volunteers, intact animals, and isolated heart and vascular tissues, isolated cardiac and vascular cells, and subcellular organelles. Our studies during FY92 are summarized below.

CARDIAC STUDIES IN HUMANS

Effects of Age and Gender on Cardiac Structure and Exercise Cardiac Performance.

To determine the independent effects of age and gender on the left ventricular (LV) structure and response to upright cycle exercise, we analyzed resting echocardiograms and gated blood pool scans at rest and maximal upright cycle exercise in 95 men and 50 women ages 23 - 86 years free of heart disease by history, physical exam, rest and exercise ECG, and if > 40 years old, exercise thallium scan. LV end-diastolic wall thickness increased with age ($r = 0.42$) and with body surface area ($r = 0.38$) in both sexes; after normalization for body size, there was no gender difference in the age-wall thickness relationship. Maximal cycle workload declined similarly with age in men (41%) and women (47%) between the third and ninth decades, although the absolute maximal load achieved was higher in men for any given age. At seated rest age-associated declines in heart rate (HR) and increases in systolic blood pressure (SBP) were observed in both sexes. Resting end diastolic volume index (EDVI) and stroke volume index (SVI) rose with age in men but not in women. At a fixed external workload of 50 watts, HR was decreased and EDVI, end-systolic volume index (ESVI), and SVI were increased with age in men but not in women. In both sexes, maximal HR, ejection fraction (EF) and cardiac index (CI) declined with age whereas ESVI and total systemic vascular resistance increased. Although EDVI at maximal effort increased with age in men but not in women, SVI was not age related in either sex. When men and women younger than 40 years who achieved similar maximal workloads of 125-150 watts were compared across workloads, women had higher HR and thence higher CIs than their male counterparts whereas the men had higher SBP responses. Fitness-matched older (> 60 years) men and women demonstrated similar exercise hemodynamics except for a higher HR response in the latter. Thus, gender has a significant influence on the age-associated changes in resting and exercise hemodynamics in normal humans. Older men appear to utilize the Frank-Starling mechanism to augment cardiac output more than older women, whereas the latter have a higher HR response. However, the apparent gender differences in LV wall thickness are eliminated by normalization for body size.

Adrenergic Regulation of Left Ventricular Volumes and Heart Rate During Stress in Healthy Humans. The response to strenuous aerobic exercise is mediated in large part by β-adrenergic activation. Age differences in the cardiac response to β-adrenergic stimulation may underlie aging changes in cardiovascular exercise hemodynamics. One way to test this hypothesis is to administer a β-adrenergic blocking drug to normal individuals across a broad age range and compare the exercise response to that in the unblocked condition. To ascertain whether acute

β-blockade would attenuate these normative age changes in cardiovascular exercise performance, we performed maximal cycle exercise in a group of the Baltimore Longitudinal Study of Aging (BLSA) men after intravenous propranolol, a β-receptor blocker. In 25 men ages 28-72 yr given intravenous propranolol, EDVI at peak workload declined with age ($r = -0.20$) causing an age-associated decline in SVI ($r = 0.48$, $p < 0.05$) not present in the unblocked men. The decline in heart with age in propranolol-treated men was blunted (0.46 beats/min/yr) compared to controls (1.09 beats/min/yr). Maximal LVEF declined with age similarly in the presence ($r = -0.50$, $p < .01$) and the absence ($r = -0.45$, $p < .0001$) of β-adrenergic blockade. The primary reason for the slope shifts in the age regressions in the presence of propranolol was a large increase in EDVI and SVI and a large decrease in HR in younger men. Thus, while the age-associated decline in maximal LVEF is unaffected, acute β-blockade reverses the age-associated ventricular dilatation during upright cycle exercise and blunts the decline in maximal HR. The net effect of β-blockade, however, is an accentuated decline of maximal CI with age.

Effect of Physical Conditioning on Cardiovascular Aging Changes in Normal Man. Because a major decline in physical conditioning status usually accompanies the aging process in industrialized societies, it is very difficult to separate the cardiovascular effects of aging per se from those of deconditioning. The following three studies seek to delineate the independent effect of these processes on LV performance, at rest and during strenuous aerobic exercise.

To determine whether intensive physical conditioning can attenuate the well-known age-associated decline in early diastolic LV function at rest, we performed resting Doppler echocardiography on 16 competitive male endurance athletes 52-76 yr, 27 age-matched sedentary men and 17 sedentary men < 40 yr old, all normal by history, physical exam and maximal treadmill ECG. Although VO_{2max} in the older athletes exceeded that in both older and younger controls (47 ± 6 vs 30 ± 7 and 41 ± 7 ml/kg/min, respectively), Doppler E velocity peak, E/A ratio and atrial filling fraction resembled those of their age-peers and indicated significant impairment of early diastolic filling compared to young control subjects. These results indicate that even high levels of aerobic training and fitness do not generally offset the age-associated decline in resting early diastolic LV function.

To determine the effect of age and conditioning status on diastolic LV performance during intense aerobic exercise, we measured radionuclide ventriculographic peak filling rates at rest and throughout graded maximal upright cycle ergometry in 56 sedentary BLSA men and 12 highly trained old men. At rest, at 50% of maximum workload and at maximal effort, peak filling rates declined with age but were similar in older athletes and their sedentary age peers. Intravenous propranolol decreased filling rates in young (37 ± 8 yr) but not in older (62 ± 6 yr) sedentary subjects. These results suggest that the age-associated decline in early diastolic filling during exercise mediated, in part, by the deficit in myocardial ß-adrenergic responsiveness with aging.

To examine the cardiovascular effects of detraining in highly conditioned older men, we performed gated radionuclide ventriculography during maximal cycle ergometry in 6 senior athletes (aged 61 ± 8 yr) before and after 12 weeks of deconditioning. Declines in VO_{2max} (51 ± 6 to 43 ± 7 ml/kg/min), SVI (66 ± 13 to 54 ± 9 ml/m^2) EF (87 ± 5 to 80 ± 4) and CI (10.2 ± 1.9 to 8.4 ± 1.2 l/μm) were noted after detraining. These results suggest that detraining reduces LV systolic performance during maximal aerobic exercise in highly conditioned older men.

Age-Associated Changes in Cardiac Rhythm and Conduction. Although respiratory sinus arrhythmia (RSA) is known to decrease with advancing age, the independent effects of age, gender, conditioning status and body composition are unknown.

The impact of age, fitness, gender and relative weight on resting HR variability was examined in 117 healthy normotensive adults ages 19-82 from the BLSA; heart rate variability was indexed by RSA, extracted from a 3-minute seated ECG using time domain digital filtering. By linear regression analysis, RSA varied inversely with age ($r = -0.61$, $p < .001$) and body mass index ($r = -0.31$, $p < .01$), directly with VO_{2max} ($r = 0.40$, $p < .001$) and was unrelated to gender. Multiple regression analysis demonstrated that age and body mass, but not VO_{2max}, were independent predictors of RSA.

Whether arrhythmias or ST segment depression detected on 24-hr ambulatory ECG have prognostic significance in asymptomatic older adults, as they do in patients with known coronary artery disease, is not known. The prognostic significance of 24-hour ambulatory ECG recordings was determined in 98 healthy BLSA volunteers > 60 years old. Over a 10 year mean followup, coronary events (CE), defined as angina pectoris, myocardial infarction or cardiac death, developed in 14 individuals. The incidence of CE did not differ between subjects who developed frequent or complex supraventricular or ventricular arrhythmias and those who did not. However, CE developed in 6 of 16 subjects with horizontal or downsloping ST segment depression versus only 8 of 82 without ST depression, $p < .05$. Thus, silent ischemia, although infrequent in apparently healthy older subjects, may be the best predictor of subsequent CE.

Almost no information is available regarding the prevalence, characteristics or prognostic significance of exercise-induced supraventricular arrhythmias. The prevalence, characteristics and prognosis of exercise-induced supraventricular tachycardia (SVT) was determined in 888 male and 555 female BLSA volunteers 17 - 94 years old. SVT occurred in 85 subjects (5.9%) and was paroxysmal atrial tachycardia in 98% of SVT episodes. SVT cases were older than those without SVT (66.0 ± 13.5 vs 49.7 ± 18.1 yr, $p < .001$) but experienced a similar incidence of subsequent CE as age-matched controls without SVT over a 5.3 year mean followup period. De novo atrial fibrillation, however, developed in 10% of SVT cases versus only 2% of controls over followup ($p < 0.10$).

MECHANISTIC STUDIES OF INTRINSIC CARDIAC MYOCYTE FUNCTION

Cellular and Subcellular Calcium Ion Homeostasis. Several projects focus on the mechanism whereby cardiac cells achieve homeostasis of Ca^{2+} ion concentration, both within the cytosol and other cellular compartments, and allow changes in Ca^{2+} in response of hormones and neurotransmitters. To determine whether mitochondrial free Ca^{2+} (Ca^{2+}_m) changes upon stimulation of rested guinea pig ventricular myocytes, we utilized our prior observations that the AM form of the fluorescent Ca^{2+} probe, indo-1 partitions both in the cytosol and in mitochondria. Assuming that indo-1 free acid (FA) localizes only in the cytosol, differences in diastolic indo-1 fluorescence ratio upon stimulation, between cells loaded with indo-1/AM and indo-1/FA, represent non-cytosolic, principally Ca^{2+}_m accumulation. During the first 35 post rest beats (at 0.5 Hz), the diastolic indo-1 fluorescence ratio increases by $32 \pm 5\%$ and $5 \pm 4\%$ (mean $\pm$ SD, n=5) in myocytes loaded with indo-1/AM and FA, respectively, indicating that a significant increase in Ca^{2+}_m begins during the initial postrest beats. This result was corroborated by a stimulation-induced increase of the steady indo-1 fluorescence ratio in AM loaded cells in which the cytosolic indo-1 fluorescence was quenched with Mn^{2+}. Thus, in guinea pig ventricular myocytes, Ca^{2+}_m is rapidly responsive to stimulation-dependent changes in cytosolic Ca^{2+}.

The response of intramitochondrial free Ca^{2+} ($[Ca^{2+}]_m$) to stimulation of isolated, single rat cardiac myocytes, both electrically and by β-adrenergic agonists was studied. Increasing frequency of electrical stimulation, over the range 0 to 2 Hz, caused modest increases in $[Ca^{2+}]_m$: β-adrenergic stimulation had no effect on the quiescent cell (mean $[Ca^{2+}]_m$ = 75 nM) but caused a marked increase in cells stimulated at 2 Hz (mean $[Ca^{2+}]_m$ increased from 120 to 570 nM). Further, when $[Ca^{2+}]_m$ was raised in non-electrically stimulated cells, e.g. by partial Na^+-

replacement, β-adrenergic stimulation had no effect. Thus, the effect of adrenergic agonists on $[Ca^{2+}]_m$ is via changes in systolic transients in Ca^{2+}, rather than on the mitochondrial Ca^{2+} transport proteins per se. Values of $[Ca^{2+}]_m$ in cells stimulated at near-physiological frequencies, viz 4 Hz, are in the range (~600 nM) giving approximately 50% stimulation of mitochondrial dehydrogenases.

Mitochondrial and Cytosolic Calcium in Cardiac Myocyte After Anoxia. Studies from this laboratory and others have documented an association between cellular Ca^{2+} loading and cardiac myocyte death following exposure to anoxia. It remains unclear whether these changes in Ca^{2+} mediate cell injury and how this may occur. We characterized the changes in Ca^{2+} which occurred in the cytosolic compartment $[Ca^{2+}]_c$ and mitochondrial compartment $[Ca^{2+}]_m$ of adult rat cardiac myocytes exposed to hypoxia/reoxygenation to define the relation between subcellular Ca^{2+} regulation and cell injury. During anoxia, $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ rose only after the onset of ATP-depletion rigor contracture. At reoxygenation 35 minutes later, $[Ca^{2+}]_c$ fell rapidly to preanoxic levels while $[Ca^{2+}]_m$ fell more slowly. Cellular hypercontracture or death was associated with a rise in $[Ca^{2+}]_c$ or $[Ca^{2+}]_m$ to greater than 250 nM just prior to reoxygenation. Reversible, relatively minor rises in $[Ca^{2+}]_m$ may be seen and do not predict cell death. The cause of cell death remains unclear but does not appear to be the result of massive reoxygenation-induced Ca^{2+} overload.

We determined whether thapsigargin, which inhibits the Ca^{2+}-dependent ATPase (the sarcoplasmic reticulum (SR) Ca^{2+} pump) in isolated SR vesicles, depletes the SR in intact rat ventricular myocytes, assessing changes in the SR Ca^{2+} content as Ca^{2+}_i transients elicited rapid, brief pulses of caffeine. After 30 minutes in 200 nM thapsigargin, responses to caffeine were abolished, indicating SR Ca^{2+} depletion. To determine whether the mode of action of thapsigargin is consistent solely with the inhibition of the SR Ca^{2+} pump, rather than a combined action on the pump and the SR Ca^{2+} release channel, we compared the effects of thapsigargin with that of ryanodine, which inhibits SR function by opening Ca^{2+} release channels. At low stimulation rates (0.2 Hz) the caffeine-dependent responses are strongly inhibited both in the presence of ryanodine and in thapsigargin. Rapid (5 Hz) pacing raises Ca^{2+}_i, providing Ca^{2+} to be available for the SR uptake. The SR Ca^{2+} loading occurs and Ca^{2+} can be released from the SR with caffeine, in the presence of ryanodine but not in thapsigargin. Thus, the mode of action of thapsigargin on the SR in situ, in intact cardiac myocytes, is consistent with an inhibition of the SR Ca^{2+} pump.

Secondary $[Ca^{2+}]_i$-Dependent Modulation of Contractility in Single Cardiac Myocytes . It is well established that the primary modes of altering myocardial contractility on a beat-to-beat basis involve both the modulation of the myoplasmic Ca^{2+} ($[Ca^{2+}]_i$) availability at the level of the myofilaments, and changes in the Ca^{2+}-response of the myofilaments, especially via length-dependent activation. Alternatively, certain more slowly developing covalent modifications of myofilaments have been recognized (such as thick or thin filament phosphorylation), but these are of uncertain functional significance in cardiac tissue. Hypothesizing that the $[Ca^{2+}]_i$ "history" regulates contractility via such covalent modification, we performed $[Ca^{2+}]_i$-clamp experiments at various levels (ranging up to 10-fold > resting $[Ca^{2+}]_i$) and durations (up to 60s) via 10 hz tetanization in SR-disabled (thapsigargin or caffeine treated) intact rat cardiac myocytes loaded with indo-1/FA. We observed that the rising phase of the tetanus (2-3 sec) was sufficiently slow such that Ca^{2+}-myofilament binding, and thus force and cell length, were throughout at equilibrium, enabling a rapid assessment of baseline contractile activation. A secondary $[Ca^{2+}]_i$- and time-dependent increase in cell shortening was observed despite steady levels of clamped $[Ca^{2+}]_i$, and without significant change in cytosolic pH, Mg or ATP (pH_i, Mg_i, ATP_i) (as assessed by seminaphthorhodafluor (SNARF) and mag-indo-1 loaded cells). This secondary leftward shift in the length-pCa curve was attenuated by the calmodulin inhibitors chlorpromazine, W7, and Sr^{2+}, and by the myosin light chain kinase (MLCK) inhibitor KT5926, and was amplified by the phosphoprotein

phosphatase inhibitor Calyculin A. We propose that $[Ca^{2+}]_i$ history, via Ca^{2+}-calmodulin-dependent MLCK activity, MLC phosphorylation, and kinase/phosphatase balance, plays a significant modulatory role in the chronic regulation of contractility in intact heart cells. This integrative phenomenon would reflect a history dependence of cardiac work/demand, and may serve as an important cardiovascular adaptive mechanism in myocardial conditioning.

Modulation of Myofilament Ca^{2+} Sensitivity as a Positive Inotropic Intervention A persistent challenge to the development of new drugs of heart failure related research has been the development of substances that increase myocardial contractility via an enhancement of myofilament responsiveness to Ca^{2+} rather than by increasing the extent of cellular Ca^{2+} loading. The diazinone derivative, EMD 53998 (designed by E. Merck, Darmstadt, Germany), increases the peak force and shift leftward the pCa-force relationship in skinned myocardial fibers; and in cell homogenates it exhibits phosphodiesterase (PDE) inhibitory activity. However, the potency of myofilament sensitization relative to that of PDE inhibition is greater than for any known substance. The effects of EMD 53998, and of its (+), EMD 57033 and (-), EMD 57439, enantiomers, were tested on the contractile properties and Ca_i transients of single, intact, guinea pig and dog cardiac myocytes. Cells were loaded with the fluorescent dye, indo-1, and bathed in a Hepes buffer at 25°C. Our aim was to ascertain whether the optical enantiomers could separate the effect mediated through PDE inhibition from that obtained via an increased myofilament responsiveness to Ca^{2+}. All three substances exerted a pronounced increase on twitch amplitude: the maximal effect of the racemate was approximately the sum of the effects of its two enantiomers. The Ca_i transient, measured as the 410/490 nm indo-1 fluorescence ratio transient, was increased by the racemate and its (-) enantiomer, but not by the (+)-enantiomer. In unstimulated cells resting length was significantly reduced by the (+)-enantiomer and this was accompanied by a decrease in indo-1 fluorescence; the (-)-enantiomer had no effect on either parameter. Qualitatively similar effects were obtained in experiments with intact dog cardiac cells. The molecular mechanism of the effect of the (+)-enantiomer was further studied in dog cardiac myofibrils. EMD 57033 stimulates the ATPase activity in myofibrils in which troponin-tropomyosin have been extracted, but does not affect Ca^{2+} binding to isolated troponin C. Furthermore, in a motility assay containing isolated actin and myosin, but devoid of Ca^{2+} and regulatory proteins, the substance increases the velocity of actin motion along myosin. Thus, in intact cells the (+)-enantiomer of EMD 53998 has direct myofilament effects which are not simply due to "Ca^{2+}-sensitization" (i.e. effects downstream to Ca^{2+}-binding to troponin C and regulatory protein modulation of thin filament activation); rather an effect on acto-myosin interaction, i.e. the cross-bridge is likely involved.

Abnormal Myocyte Ca^{2+} Handling in A Rodent Model of Chronic Heart Failure. Compensated myocardial hypertrophy occurring in response to chronic pressure overload usually progress to a decompensated state, with myocardial depression and cardiac pump dysfunction. The mechanisms involved in the transition from the chronically adapted to the chronically failing heart remain unclear. A major reason has been a relative lack of animal models of stable hypertrophy. We have developed a practical and reproducible model of cardiac hypertrophy that progresses to chronic heart failure, within three months, by banding the ascending aorta of young rats. Gradual LV overload occurs as the animals mature, leading to a compensated LV hypertrophy and subsequent cardiac failure. Animals with cardiac failure, identified by clinical symptoms (increased respiratory rate), presented pathophysiological alterations consistent with heart failure: high lung weight, hypertrophy of the left atria, right ventricle, and right atria. In addition most of the failure animals were observed to have pleural effusions. The first physiological study on animals provided by our experimental model shows that the contractile behavior of myocytes isolated from failing and control hearts tended to be different under stressful conditions. Myocytes from failing hearts had reduced rates of twitch shortening and rates of relengthening, especially at high drive rates (2.5 Hz) or at elevated external Ca^{2+}

concentrations (6 mM), compared to control. Application of the different technology available in the laboratory, such as measures of intracellular Ca^{2+} handling with Indo-1, molecular biology ..., to this model of progressive heart failure will be utilized to elucidate mechanisms that underlie the transition from compensated hypertrophy to chronic heart failure.

Membrane Ion Transport Mechanisms. Both Na,K-ATPase and Ca-ATPase are critical cation transport systems in membranes of eucaryotic cells. The plasma membrane Na,K-ATPase pumps Na^+ out of cells in exchange for K^+. SR Ca-ATPase is important for lowering intracellular Ca^{2+} in the heart between contractions. Increasing our insight into the mechanism and the regulation of these enzymes is of widespread interest at the molecular, the membrane, the cellular, and the whole animal level. The most fundamental questions involve 1) the free energy coupling between ATP hydrolysis and ion transport, 2) the molecular structure of the ion channels, and 3) the regulation of enzyme activity to meet the needs of the cell and the organism. Insight into these mechanisms may be crucial for our understanding of disease and aging.

In a series of experiments, we have gained insight into the nature and mechanism of the three Na^+ binding sites within the ion channel of the Na,K-ATPase. A model for Na^+ transport by electric organ Na,K-ATPase is proposed based on the statistical partitioning of Na^+ binding states. The model accounts for (1) the biphasic time course of phosphorylation obtained under conditions where Na^+ is added last, and (2) the conversion to monophasic kinetics when the enzyme is preincubated with Na^+ prior to ATP addition. These results imply that the third Na^+ binding site is restricted when the other two sites are occupied.

In other experiments we examined the coupling of Ca transport to ATP hydrolysis in isolated cardiac SR. Quenched-flow measurements of phosphoenzyme formation by the Ca^{2+} pump in SR were performed at 2°C for comparison with EPR (electron spin resonance) spectral data demonstrating a slow conformational change associated with active Ca^{2+} transport. Phosphoenzyme formation was biphasic with a close correlation between the slow phase of phosphorylation and the EPR spectral change. The results indicate that the spin label senses a protein conformational change linked to phosphorylation of the Ca^{2+}-ATPase or to the interconversion of subsequently-generated phosphorylated intermediate states. These studies provide important information about the mechanism for free energy coupling between ATP hydrolysis and Ca^{2+} transport.

REGULATION OF ENERGY METABOLISM IN AGING AND DISEASE

Regulation of Energy Metabolism in Aging and Disease: Cardiovascular System. This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise tissue survival. Experimental results derive from two paradigms relating to heart failure and death of cardiac myocytes. (1) We have investigated a changed $[Ca^{2+}]_m$ as a possible mechanism of the less active pyruvate dehydrogenase which we identified in 2 strains of hamster with an inborn cardiomyopathy (B10 14.6 and TO.2) and described in last year's report. Work with isolated mitochondria showed similar gradients of Ca^{2+} across the mitochondrial membrane in myopathic and healthy strains - i.e. no intrinsic change in the mitochondrial Ca^{2+}-transport proteins *per se*. However, measurements of $[Ca^{2+}]_m$ *in situ* in isolated cardiac myocytes from myopathic animals showed lower values compared to healthy animals, thought to be due to lower transients in cytosol $[Ca^{2+}]$ on electrical excitation. These results provide a possible mechanism for the impaired dehydrogenase regulation in this animal. (2) Mitochondrial function has been evaluated in isolated cardiac myocytes using the newly-described fluorescent dye JC-1, which reports on mitochondrial membrane potential ($\Delta\psi$). De-energization of the cells due to anoxia or respiratory chain inhibition allows some remaining measure of contractile function and preservation of $\Delta\psi$. Additional inhibition of glycolysis leads to collapse of both parameters. Evidently ATP derived from

glycolysis can sustain $\Delta\psi$, under the experimental conditions employed, through a reversal of the mitochondrial protontranslocating ATPase.

Regulation of Energy Metabolism in Aging and Disease: Central Nervous System. This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise survival of neurones. We have examined the possibility that impaired mitochondrial protein synthesis may result in decreased respiratory chain function in old age. The respiratory chain complexes chosen for study are those in which one or more subunit is coded for by mT-DNA; viz, NADH-CoQ oxidoreductase (Complex 1); cytochrome c oxidase (Complex IV); F1-Fo-ATPase (Complex V). In addition, State 3 (ADP-dependent) O_2-uptake with glutamate plus malate and with succinate as substrate was studied. At this time, activities have been found to be similar in cerebral cortex, hippocampus and striatum, when young adult (6 mo) and senescent (24 mo) male rats were compared. In collaborative studies with the Laboratory of Biological Chemistry, we have also initiated a study of the incidence of deletions of mt-DNA in the brain regions identified above, as a function of aging.

MECHANISMS OF ACUTE RECEPTOR MEDIATED MODULATION OF CARDIAC CELL PERFORMANCE

Mechanisms of Signal Transduction of Opioid Receptor Stimulation of Myocytes. The recent discovery of the presence of opioid peptide receptors on cardiac ventricular cells has prompted investigation of functional effects that result from stimulation of these receptors. In this regard, a naturally occurring opioid peptide, leucine enkephalin, a δ receptor agonist, leads to a marked reduction in the twitch amplitude of single adult rat ventricular myocytes. This effect is due, in large part, to a reduction in the amplitude of the cytosolic Ca^{2+} transient (Ca_i). The Ca_i transient following the excitation of heart cells is due to activation of L-type sarcolemmal Ca^{2+} channels leading to Ca^{2+} influx via these channels (I_{Ca}). This Ca^{2+} influx triggers Ca^{2+} release from the SR and also loads the SR with Ca^{2+} for subsequent releases. The specific mechanism by which Ca_i is reduced by leucine enkephalin have been partly elucidated. Leuenk causes a release of Ca^{2+} from the SR and leads to a reduction in the amount of Ca^{2+} in the SR stores. These effects may be attributable to an increase in IP_3 and IP_4 produced by leucine enkephalin. However, it is unknown whether Leuenk also decreases I_{Ca}. In this regard, the effect of opioid peptides to block neurotransmitters release from neurons has been attributed to a reduction in Ca^{2+} channel current in these cells. In the present study we determined the effect of leucine enkephalin on I_{Ca} of individual cardiac ventricular cells freshly isolated from adult rats. I_{Ca} was measured via patch pipette in the whole cell voltage clamp mode. We observed that leucine enkephalin (10^{-7}M) decreases the amplitude of I_{Ca} by 40% during regular stimulation at 0.2 Hz at 23°C. Thus, the I_{Ca} IV relation was not altered by leucine enkephalin. The I_{Ca} depression by leucine enkephalin was abolished by Naloxone, a specific δ receptor antagonist. The I_{Ca} inactivation kinetics were unaffected by leucine enkephalin. That leucine enkephalin decreases the magnitude of I_{Ca} indicates that stimulation of δ opioid receptors leads to both a reduction in the magnitude of the trigger for Ca^{2+} release and to a reduction in Ca^{2+} loading of the SR. Thus, the opioid peptide effects to decrease the Ca_i transient and contraction amplitudes in individual cardiac ventricular cells, are, in part, due to a reduction in I_{Ca}.

α-Adrenergic Stimulation in Myocardial Cells. We have elucidated several aspects of the mechanisms of myocardial α_1-adrenoceptor (α_1-AR) stimulation were examined: (1) The positive inotropic action of α_1-adrenoceptor stimulation, at least in part, is due to an enhanced myofilament responsiveness to Ca^{2+} mediated by activation of Na^+/H^+ exchange and an increase in pH_i. We tested the hypothesis that the increase in pH_i may be due to protein kinase C (PKC)-mediated activation of Na^+/H^+ exchange. In experiments with isolated rat myocytes α_1-AR stimulation with phenylephrine and nadolol enhanced contraction and pH_i.

However, following PKC inhibition with staurosporine or downregulation with overnight exposure to a tumor-promoting phorbol ester, α_1-AR stimulation had no effect on pH_i and decreased twitch amplitude. Thus, PKC-mediated activation of Na^+/H^+ exchange increases pH_i and contributes to the positive inotropic action of α_1-AR stimulation. In contrast, inhibition of PKC-dependent effects makes apparent a negative inotropic effect of α_1-AR stimulation. (2) We next examined the effect of α_1-AR subtypes, α_{1A} and α_{1B} on contraction, Ca_i and myofilament response to Ca^{2+} of isolated myocardial cells. α_{1A}-AR stimulation (phenylephrine, nadolol and α_{1B}-AR inactivation with chloroethylclonidine) increased contraction, Ca_i transient amplitude and myofilament response to Ca^{2+}. In contrast α_{1B}-AR stimulation (phenylephrine, nadolol and α_{1A}-blockade with WB-4101) decreased contraction, Ca_i transient amplitude and downregulated the α_{1A}-AR effect to increase myofilament response to Ca^{2+}. (3) Myocardial damage and arrhythmias due to reperfusion after ischemia are worsened by α_1-AR stimulation and improved by acidosis. We examined the effect of α_1-AR on pH_i and aftercontractions during recovery from hypercarbic acidosis. α_1-AR increased pH_i and the frequency of aftercontractions upon recovery from acidosis. Both effects were prevented by ethylisopropylamiloride (EIPA), a blocker of Na^+/H^+ exchange. Under similar experimental conditions α_{1A}-AR stimulation enhanced and α_{1B}-AR stimulation markedly decreased the frequency of aftercontractions over the effect on non-selective α_1-AR stimulation.

Comparison β_2 vs β_1 Adrenoceptor Stimulation in Rat Cardiocytes. β_1- and β_2-adrenoceptors (β_2-AR and β_1-AR) exist in the heart. While the cellular effects of the former are widely studied, the specific effects of the later have not been elucidated. We studied the effects of β_2-AR and β_1-AR agonists on the Ca_i transient, indexed by the transient increase in indo-1 fluorescence ratio following excitation, twitch amplitude (TA), measured via photodiode array, and membrane potential and L-type sarcolemmal Ca^{2+} current, and I_{Ca}, measured by whole cell patch electrode in single rat ventricular myocytes. The selective β_2-AR agonist Zinterol increased the amplitudes of both the Ca_i transient and twitch in a concentration-dependent manner. Similar results were obtained when β_2A-R were stimulated with isoproterenol in the presence of the selective β_1-AR antagonist, CGP 20712A (CGP). β_1-AR stimulation induced by norepinephrine (NE) increased TA to about the same extent as β_2-AR. However, several striking differences between response to β_1-AR and β_2-AR stimulation were observed. β_1-AR stimulation had a potent effect to abbreviate the time course of the twitch contraction and Ca_i transient; β_2-AR stimulation did not reduce the time course of the Ca_i transient and had only a minor effect on the twitch duration. For a given increase in TA, β_1-AR stimulation caused a greater increase in Ca_i transient, suggesting a diminished Ca_i-myofilament interaction. β_1-AR, but not β_2-AR stimulation, evoked spontaneous Ca_i oscillations, increased the diastolic indo-fluorescence level and caused a decline in resting cell length. β_1-AR and β_2-AR also differed in their effects on I_{Ca}. While both β_1-AR and β_2-AR stimulation increased the peak I_{Ca} amplitude, β_2 markedly prolonged the I_{Ca} inactivation time. Accordingly, β_2-AR stimulation prolonged the action potential duration to a greater extent than β_1-AR stimulation did. CPT cAMP mimicked the effects of β_1-AR stimulation by NE but not those due to β_2-AR stimulation. These results clearly indicate that both β_2-AR and β_1-AR functionally coexist in rat ventricular myocytes, but that stimulation of these receptor subtypes elicits qualitatively different cell responses at the levels of ionic channels, the myofilaments, and SR.

Mechanism of Contractile Deficit of Rat Heart Cells to Norepinephrine With Aging. In both humans and animals the effectiveness of ß-adrenergic stimulation to augment the heart contraction strength declines with aging. We have investigated cellular mechanisms that may underlie the aging deficit. TA, (%rest cell length), measured via photodiode array and Ca_i transient, indexed as transient in Indo-1 fluorescence ratio 410/940 μm (IF) were measured in single ventricular myocytes of rats of varying age during electrical stimulation at 23°C. The L type Ca^{2+} current (I_{Ca}, pA/pF) was measured in additional cells during voltage clamp from -40 to 0 mV for 200 msec at 0.5 Hz (CsCl, 120, ATP, 3, and EGTA 10 mM in pipette; CsCl 5, and Ca 1 mM in bath). With aging the maximum TA, IF and I_{Ca} response to NE (10^{-7}M) are blunted.

	A (n=85), each age)		IF (n=85, each age)		I_{Ca} (n=20, each age)	
	Control (C)	% C NE**	Control (C)	% C NE*	Control (C)	% C NE**
3 Mo	6.27±0.32	222.73±9.33	0.200±0.009	193.77±10.44	5.32±0.53	235.83±20.19
8 Mo	6.71±0.30	174.38±7.66	0.225 ±0.012	170.17±14.10	5.36±0.42	179.46±12.79
24 Mo	6.63±0.44	139.00±9.98	0.260±0.012	144.36±10.38	5.33±0.61	156.71±14.07

Age effect *$p<0.02$; **$p<0.001$.

Thus, the blunted TA and CaT responses to NE with aging are due to a failure to sufficiently augment Ca^{2+} influx via I_{Ca} to trigger Ca^{2+} release from the SR and to Ca^{2+} load the SR.

CARDIAC AND SKELETAL MUSCLE GENE EXPRESSION

Involvement of Cardiac Opioids in Response of the Heart to Stress. The magnitude of cardiac hypertrophy is altered with advancing age and is accompanied by an increase in cardiac opioid peptide production. Previous work in the laboratory has shown that opioid peptides can directly modulate cardiac myocyte contractile function and inhibit catecholamine induced inotropy by affecting signal transduction through the β-adrenergic pathway. Since cardiac hypertrophy is believed to be a regulated in part by the β-adrenergic signal transduction pathway, we hypothesized that cardiac opioids regulate the hypertrophic process. The objectives of this study were to determine whether the cardiac response to hypertrophic stress is associated with changes in opioid mRNA and peptide levels in rat heart, and whether opioid gene expression in the heart is mediated by adrenergic stimulation. Cardiac opioid production was upregulated 2-3-fold with advancing age, and proenkephalin gene expression was elevated in the heart during aging (7-fold), and during chronic pressure overload hypertrophy leading to heart failure (2-fold). Paradoxically, proenkephalin gene expression is transiently downregulated after 3 days of aortic constriction, and this transient decrease was abolished by α-adrenergic blockade. Results of studies on cultured neonatal cardiac myocytes indicate that: (1) proenkephalin mRNA abundance is increased in myocyte cultures supplemented with NE, peaking at 4 hours after addition, (2) α- and β-adrenergic receptor stimulation act synergistically to increase the expression of proenkephalin, and (3) myocyte cultures of high purity permit the induction of hypertrophy marker genes but not proenkephalin by NE treatment, while the opposite is true for cultures containing cardiac fibroblasts. The mutually exclusive expression of proenkephalin and markers of hypertrophy

suggests that opioid peptides may inhibit cardiac myocyte hypertrophy. In addition, the level of proenkephalin induction achieved in mixed cultures by NE treatment is sufficiently higher than that in cultures of cardiac fibroblasts, suggesting that a myocyte-fibroblast interaction exists which enhances the proenkephalin expression in one or both of these cell types.

Age-Associated Patterns of Gene Expression in the Heart. Atrial natriuretic factor (ANF) is synthesized and secreted by atrial tissue in response to experimental or genetic hypertension. Recent studies have shown that ANF is produced and secreted by LV tissue as well, and that ANF has direct effects on ventricular cardiac myocyte contraction. Since heart contractile function is altered during aging, and the senescent heart exhibits many other phenotypic and genotypic characteristics of the hypertensive heart, we tested the hypothesis that the ANF gene would be upregulated with advancing age. In addition we studied differences in the response of ANF mRNA levels to aortic constriction in hearts of young adult and senescent rats. Total RNA was isolated from LV of male Wistar and Fischer rats aged 1.5-27 mo of age. Northern blots were probed with a radiolabeled cDNA probe synthesized by PCR using oligonucleotides complementary to the published sequence. The levels of mRNA coding for ANF increased progressively with advancing age in ventricles of both strains of rats. ANF mRNA abundance was 7-fold greater in ventricles of senescent compared to young adult rats. In freshly isolated ventricular myocytes, a similar pattern was observed. The induction of the ANF gene in ventricles of aortic-constricted rats was blunted with advancing age. Since ANF peptide is known to affect cardiomyocyte function, the age-associated increase in ANF gene expression may be of functional importance in the senescent heart.

Cardiac Gene Expression During the Transition from Hypertrophy to Heart Failure. The mechanism(s) which contribute to the transition from compensated cardiac hypertrophy to heart failure have remained obscure. Our purpose was to determine whether some of the changes observed during the transition to heart failure might be regulated at the level of gene expression. The spontaneously hypertensive rat (SHR) served as a model for these studies. This model has recently been characterized in terms of functional and morphological features. At 18 mo of age some SHR rats develop clinical signs and symptoms of failure. By combining daily inspections with post-mortem morphological and pathological data, the SHRs can be divided into those exhibiting failure and those in whom cardiac function is well compensated. Since the failing heart is characterized by impaired function and increased fibrosis, we studied levels of contractile protein and connective tissue mRNA. Total RNA was isolated from hearts of 18-24 mo normotensive Wistar-Kyoto (WKY) rats, and SHRs with (F) and without (NF) evidence of heart failure. Relative mRNA levels for ANF, fibronectin, α-skeletal and α-cardiac actin, and α- and β-myosin heavy chain (MHC) were assessed. The transition from compensated hypertrophy to failure was accompanied by decreased contractile protein gene expression (α-MHC; 28% of SHR-NF value) and increased expression of fibronectin (301% of SHR-NF value). Expression of ANF was maintained at an elevated level compared to that of age-matched WKY (365%). The results suggest that the impaired function and increased fibrosis observed in failing hearts may be regulated, in part, at the level of gene expression.

MyoD1 and Developmental Gene Expression in Skeletal Muscle. The (skeletal) muscle regulatory factors (MRF), of which myoD1 is the prototypical example, are

a family of helix-loop proteins which are functionally united by their ability to direct cells from diverse developmental and tissue origins to the skeletal muscle lineage. These factors, however, also act as transcription factors for a subgroup of skeletal muscle-specific genes and exhibit different developmental patterns of expression. To examine this possibility and to elucidate the mechanism(s) by which this could occur, we have chronicled the changes in MRF and muscle isoform expression that occur in chicken skeletal muscle at specific stages in development, concentrating on the transition between embryonic and fetal stages. This transition involves the switching of contractile protein isoforms from the "cardiac" to the "skeletal" type, occurs in concert with increases in nerve-muscle activity, and is associated with increased PKC activity. During this period, the level of MRF mRNA for myoD1 and myogenin is also reduced. In noninnervated cultures of skeletal muscle cells, the level of these same MRF can be reduced by either pharmacologically or genetically elevating PKC activity. Significantly, just as during the embryonic to fetal transition, the levels of "cardiac" type isoforms are also reduced. All of these genes possess transcriptional regulatory sequences that are targets for myoD1 and myogenin binding. These results suggest that one of the mechanisms responsible for the changes in gene expression during the embryonic to fetal transition involves downregulation of MRF expression in response to elevated PKC activity. This, in turn, results in transcriptional silencing of a group of genes which utilize the MRF as obligatory transcription factors. The time course of these events suggest that the effect of PKC on MRF expression may be direct.

LCS VASCULAR INITIATIVE

Age-Associated Changes in Vascular Stiffness Properties. As arterial and myocardial changes occur in otherwise healthy older individuals within the Western society they are often referred to as a part of a "normal aging process." However, the arterial stiffness increase (indexed by an increase in arterial pulse wave velocity) and the arterial pressure increase with age are heterogeneous among various populations worldwide and among individuals within a given population. The ultimate goals of this project are to address the issue of how alterations in arterial stiffness affect the myocardium and whether in outcome studies they relate to vascular insufficiency syndromes, e.g., stroke. We have initiated a pilot study in which measurements of cardiac mass (via NMR), filling properties and isovolumic relaxation time (via Doppler echocardiography), carotid pressure pulse (via applanation tomography) and arterial pulse wave velocity (Doppler sonography) are made in men and women who differ with respect to age, arterial pressure, body composition, and physical conditioning status. As expected, results indicate that with increasing age arterial stiffening leads to an increase in pulse wave velocity. This is associated with an early return of reflected pulse waves from peripheral sites which produce an augmented and late occurring peak of carotid pressure pulse. It is important to note that these arterial changes are, by and large, not detected by routine clinical measures of brachial arterial pressure and are independent of gender but vary inversely with exercise capacity. Of note, also is that in highly physically conditioned older individuals (>60 yr of age) the arterial stiffness and reflected wave indices are markedly reduced and do not differ from those of younger individuals, although the age-associated increase in systolic pressure persists. We have taken the initiative to extend many of these studies to Black

Americans and to other non-Western populations in which different patterns of arterial pressure change occurs with aging (in China via a research contract).

Mechanisms for Signal Transduction of Shear Stress Forces in Endothelial Cells. Wall shear stress in blood vessels plays a role in the development of restenosis after angioplasty, coronary artery bypass graft occlusion and atherosclerosis. In addition these processes are also modulated by the interaction of vascular cells with the endothelium. The purpose of this project is: (1) To characterize, in endothelial cells (EC), the effect of acute changes in shear stress forces on cytosolic pH $(pH)_i$ and $[Ca^{2+}](Ca_i)$. (2) To determine the effects of changes in Ca_i on EC pH_i and 3) to determine how the interaction between the endothelium and other cells affects Ca_i homeostasis in EC. The effect of laminar shear stress on EC pH_i was studied by examining EC cultured in glass capillary tubes. Shear stress forces led to a rapid decrease in EC pH_i in the presence of HCO_3^-. This response was markedly blunted by the anion exchange inhibitor 4-acetamido,4'-isothio-cyanatostilbene-2,2'-disulfonic acid (SITS) but unaffected by Na^+ removal. In the absence of HCO_3^-, shear stress forces caused a small increase in pH_i which was abolished by ethylisopropylamiloride (EIPA), a Na^+/H^+ exchange inhibitor. The effect of acidification on Ca_i was examined in the absence of significant shear stress forces either by removal of NH_4Cl, changing from a bicarbonate-free to a 5% CO_2/HCO_3^--buffered solution at constant buffer pH, or changing from a 5% CO_2/HCO_3^- to a 20% CO_2/HCO_3^- solution. Regardless of the method employed, intracellular acidification resulted in an increase in Ca_i indexed by the fluorescent Ca^{2+} indicator indo-1. The increase in the indo-1 fluorescence ratio induced by changing from a 5% CO_2/HCO_3^- to a 20% CO_2/HCO_3^- solution was not significantly altered by removal of buffer Ca^{2+} either before or after depletion of bradykinin-sensitive intracellular Ca^{2+} stores. In other experiments we examined the effect of leukocyte adhesion on EC Ca_i. Upon contact between granulocytes or monocytes with EC there was a rapid increase in EC's Ca_i which exhibited a partial recovery toward control. This response was abolished after depletion of EC endoplasmic reticulum Ca^{2+} with thapsigargin while it was not affected in a Ca^{2+}-free buffer. Similar results were obtained when melanoma cells were used in place of leukocytes. EC contact with 8 μm inert beads did not elicit an increase in Ca_i. Thus, in vascular EC, shear stress forces activate both an alkali extruder, Na^+-independent Cl^-/HCO_3^- exchange, and to a smaller extent an acid extruder, Na^+/H^+ exchange; the net effect in a physiologic bicarbonate buffer is a decrease in pH_i. The results indicate that intracellular acidification of vascular EC releases Ca^{2+} into the cytosol either from pH-sensitive intracellular buffer sites, mitochondria, or from bradykinin-insensitive intracellular stores. This Ca^{2+} mobilization may be linked to endothelial synthesis and releases of vasodilatory substances during acidosis.

Angiogenesis Endothelial Cell Function and Aging. Angiogenesis (the formation of new blood vessels) is an important process in tumor growth, wound healing, retinopathies, and rheumatoid arthritis. We are investigating aspects of angiogenesis in an in vitro model utilizing isolated human and bovine endothelial cells and a mixture of basement membrane matrix proteins (Matrigel). Plating EC on Matrigel-coated tissue culture dishes led to their rapid (12 to 18 hr) organization into a capillary-like network.

Normally EC are quiescent *in vivo*; but in response to an angiogenic factor, activated EC will breakdown their underlying basement membrane, migrate into the

interstitium, proliferate, and finally differentiate into a new blood vessel. We found that quiescent EC (contact inhibition or serum starvation) were not able to organize into a capillary-like network in vitro, while cells released from contact inhibition or feed serum-containing media regained the ability to organize. There was a correlation between the ability to organize on Matrigel and the expression of cell surface receptors for the basement membrane proteins, laminin and collagen IV. These studies should prove useful in studying some early events involved in angiogenesis.

Often angiogenic factors attract leukocytes and fibroblast, while stimulating the formation of new blood vessels. Previous studies have demonstrated that leukocytes can release angiogenic factors, but it is unclear whether fibroblast cells may also be involved. We tested whether fibroblasts may produce factors that alter EC function. We found that fibroblast-conditioned media increased EC proliferation, contained a cellular attractant, and enhanced the extent of capillary-like formations on the Matrigel. The ability of fibroblasts to influence angiogenesis may be an important component of wound-healing.

Metabolism Affects Calcium in Cultured Aortic Endothelial Cells. Hypoxia is known to affect both EC adenine nucleotide metabolism and release of vasoactive substances, but the link between these is unknown. Since the release of these vasoactive substances may be in part mediated by an increase in $[Ca^{2+}]_i$ we investigated the effects of metabolic inhibitors on $[Ca^{2+}]_i$ in indo-1 loaded cultured rat aortic EC. Inhibition of oxidative phosphorylation (NaCN, 2 mM) or substrate deprivation alone (no glucose) caused little change in $[Ca^{2+}]_i$. In contrast, combined inhibition of oxidative phosphorylation and substrate deprivation (NaCN, no glucose) caused a significant increase in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ rose similarly even when cells were studied in the absence of external Ca^{2+}. The greatest increase in $[Ca^{2+}]_i$ occurred in the absence of substrate and during exposure to an inhibitor of glycolysis (iodoacetate, 2 mM). These results suggest a lack of endogenous oxidative substrate and a critical dependence on glycolytic metabolism.

Vascular Cell Function and Aging. Aging constitutes a risk factor in the development of cardiovascular disease. Older animals are more susceptible to vascular lesions after endothelial injury than are young animals. The mechanism leading to the increased proliferation of smooth muscle cells (SMC) in the intima is unknown. However, the intimal thickening may result from excessive production of growth factors and/or increasing the activities of proteinases known to enhance the degradation of the extracellular matrix components. The purpose of these studies was to examine the factors involved in the development of intimal thickening. Histological staining of the thoracic aorta with TGFβ specific antibodies have revealed the presence of higher levels of extracellular TGFβ_1 in the basement membranes from old rats as compared to young. In contrast, no apparent age-related differences were detected in the levels of intracellular TGFβ_1 or extracellular TGFβ_2 and TGFβ_3. Significant elevations in collagenase activity were detected in aortic homogenates from old and balloon injured animals. These elevations may be involved in the degradation of matrix protein associated with SMC migration and intimal thickening.

Age-related alterations in smooth muscle ion metabolism may contribute to the onset and progression of hypertension. A model for the investigation of this

process involves freshly-isolated single rat arterial SMC. These cells were used to investigate the relationship between intracellular [Ca^{2+}] fluctuations and initial velocity of cell shortening induced by norepinephrine and K^+ depolarization. The results showed that single cells retain a Ca^{2+}-dependent variation in shortening velocity consistent with the behavior observed in multicellular (tissue) preparations.

Role of Vascular Smooth Muscle Cells in Vascular Disease. The development and progression of several vascular diseases depend on the migration and proliferation of vascular SMC (VSMC) and their interaction with extracellular matrix. We have found previously that this interaction is due in part to the invasion of reconstituted basement membrane (Matrigel) by VSMC in response to PDGF. Proliferative (dedifferentiated) VSMC exhibit fivefold greater invasiveness as compared with post-confluent (differentiated) VSMC. We have demonstrated that intracellular Ca^{2+} dynamics play an important role in VSMC invasion but not in chemotaxis. Chemotaxis reflects the ability of cells to attach to a substrate and migrate in response to a chemoattractant. Chemoinvasion requires the additional ability of cells to degrade a barrier. We have demonstrated 72 KD type IV and 92 KD type IV collagenase activity by zymography in invasive VSMC. Northern blot analyses have indicated mRNA for 72 KD type IV collagenase but not 92 KD type IV collagenase in these cells. Invasive VSMC also express mRNA for the tissue inhibitors of matrix metalloproteinases (TIMPs) - reflecting the importance of a balance between positive and negative regulators. Chemoinvasion assays employing antiserum to the 72 KD type IV collagenase, nonimmune serum, and antiserum to the 92 KD type IV collagenase, have demonstrated that VSMC invasion in response to PDGF is mediated by the 72 KD type IV collagenase. Chemotaxis is less dependent on the 72 KD type IV collagenase. Because these collagenases are secreted in zymogen form, activation is required for degradation of ECM. Experimental results employing BAPTA (a chelator of intracellular Ca^{2+}) and ionomycin (a Ca^{2+} ionophore) suggest Ca^{2+} may be important in this activation.

Vascular Smooth Muscle Gene Expression and Cellular Differentiation. The phenotypic modulation of VSMC from a quiescent, differentiated state to that of proliferating, "undifferentiated" state is a common pathogenic feature of vascular disease. We had previously shown that proliferating VSMC express a mRNA with strong homology to the mouse ID (Inhibitor of Differentiation) gene and this expression was downregulated when these cells differentiated. In skeletal muscle, the product of the ID gene is thought to acta as a transdominant suppressor of the muscle regulatory/transcription factors that belong to the helix-loop-helix (HLH) family of proteins. Expression of ID in proliferating skeletal muscle precursor cells and its downregulation preceding differentiation prevents HLH muscle transcription factors from activating skeletal muscle-specific gene expression in the proliferating precursor cells. Using a variety of molecular cloning approaches, we have identified and cloned the cDNAs for three putative ID-like proteins that are expressed in proliferating VSMC. We have identified one of these as rat ID, the rate homologue of the original mouse ID cDNA. Rat ID is expressed at low levels in the vessels of mature rats, but at very high levels in proliferating VSMC derived from these vessels. Its expression is significantly reduced by conditions that bring about growth arrest of the cells, such as confluence, serum withdrawal, or contact with basement membrane matrices. VSMC cultures established from the vessels of older rats also

express high levels of rat ID as they proliferate, but unlike "younger" VSMC do not downregulate ID levels in response to those manipulations that lead to growth arrest in the "younger" cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00226-10 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitation-Contraction Mechanisms in Isolated Cardiac Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	A. M. Janczewski	Visiting Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. To determine whether mitochondrial free Ca^{2+} (Ca^{2+}_m) changes upon stimulation of rested guinea pig ventricular myocytes, we utilized our prior observations that the AM form of indo-1 partitions both in the cytosol and in mitochondria. Assuming that indo-1 free acid (FA) localizes only in the cytosol, differences in diastolic indo-1 fluorescence ratio (IFR) upon stimulation, between cells loaded with indo-1/AM and indo-1/FA, represent non cytosolic, principally Ca^{2+}_m accumulation. During the first 35 post rest beats (at 0.5 Hz), the diastolic IFR increases by 32 ± 5% and 5 ± 4% (mean ± SD, n=5) in myocytes loaded with indo-1/AM and FA, respectively, indicating that a significant increase in Ca^{2+}_m begins during the initial postrest beats. This result was corroborated by a stimulation-induced increase of the steady IFR in AM loaded cells in which the cytosolic indo-1 fluorescence was quenched with Mn^{2+}. Thus, in guinea pig ventricular myocytes, Ca^{2+}_m is rapidly responsive to stimulation-dependent changes in cytosolic Ca^{2+}.

B. We determined whether thapsigargin (TG), which inhibits the Ca^{2+}-dependent ATPase (the SR Ca^{2+} pump) in isolated SR vesicles, depletes the SR in intact rat ventricular myocytes, assessing changes in the SR Ca^{2+} content as Ca^{2+}_i transients elicited rapid, brief pulses of caffeine. After 30 min in 200 nM TG, responses to caffeine were abolished, indicating SR Ca^{2+} depletion. To determine whether the mode of action of TG is consistent solely with the inhibition of the SR Ca^{2+} pump, rather than a combined action on the pump and the SR Ca^{2+} release channel, we compared the effects of TG with that of ryanodine (RY), which inhibits SR function by opening Ca^{2+} release channels. At low stimulation rates (0.2 Hz) the caffeine-dependent responses are strongly inhibited both in the presence of RY and in TG. Rapid (5 Hz) pacing raises Ca^{2+}_i, providing Ca^{2+} available for the SR uptake. The SR Ca^{2+} loading occurs and Ca^{2+}_i can be released from the SR with caffeine, in the presence of RY but not in TG. Thus, the mode of action of TG on the SR *in situ*, in intact cardiac myocytes, is consistent with an inhibition of the SR Ca^{2+} pump.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00228-09 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Changes in Cardiac Rhythm and Conduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. Fleg	Cardiologist	LCS, NIA
Others:	P. Vaitkevicius	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

University of Maryland, College Park (E. Byrne, S. Porges); Rush Medical College, Chicago (Harold Kennedy).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Although respiratory sinus arrhythmias (RSA) are known to decrease with advancing age, the independent effects of age, gender, conditioning status and body composition are unknown. The impact of age, fitness, gender and relative weight on resting heart rate variability was examined in 117 healthy normotensive adults ages 19-82 from the BLSA; heart rate variability was indexed by RSA, extracted from a 3-minute seated ECG using time domain digital filtering. By linear regression analysis, RSA varied inversely with age ($r = -0.61$, $p < .001$) and body mass index ($r = -0.31$, $p < .01$), directly with VO_{2max} ($r = 0.40$, $p < .001$) and was unrelated to gender. Multiple regression analysis demonstrated that age and body mass but not VO_{2max} were independent predictors of RSA.

B. Whether arrhythmias or ST segment depression detected on 24-hr ambulatory ECG have prognostic significance in asymptomatic older adults, as they do in patients with known coronary artery disease, is not known. The prognostic significance of 24-hr ambulatory ECG recordings was determined in 98 healthy BLSA volunteers > 60 yr old. Over a 10 yr mean followup, coronary events (CE), defined as angina pectoris, myocardial infarction or cardiac death, developed in 14 individuals. The incidence of CE did not differ between subjects who developed frequent or complex supraventricular or ventricular arrhythmias and those who did not. However, CE developed in 6 of 16 subjects with horizontal or downsloping ST segment depression versus only 8 of 82 without ST depression, $p < .05$. Thus, silent ischemia, although infrequent in apparently healthy older subjects, may be the best predictor of subsequent CE.

C. Almost no information is available regarding the prevalence, characteristics or prognostic significance of exercise-induced supraventricular arrhythmias. The prevalence, characteristics and prognosis of exercise-induced supraventricular tachycardia (SVT) was determined in 888 male and 555 female BLSA volunteers 17-94 yr old. SVT occurred in 85 subjects (5.9%) and was paroxysmal atrial tachycardia in 98% of SVT episodes. SVT cases were older than those without SVT (66.0±13.5 vs 49.7±18.1 yr, $p < .001$) but experienced a similar incidence of subsequent cardiac events as age-matched controls without SVT over a 5.3 yr mean followup period. De novo atrial fibrillation, however, developed in 10% of SVT cases versus only 2% of controls over followup ($p < 0.10$).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00231-08 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Energy Metabolism in Aging and Disease: Cardiovascular System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R. G. Hansford	Chief, EMBS	LCS, NIA
	F. Di Lisa	Visiting Associate	LCS, NIA
Others:	B. Hogue	Chemist	LCS, NIA
	I. Kudryashova	Visiting Fellow (EOD 10/91)	LCS, NIA

COOPERATING UNITS (if any)

Cardiac Function Section, LCS; Division of Cardiology, Johns Hopkins University (R. Weiss, H. Silverman, M. D. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise tissue survival. Experimental results this year derive from two paradigms relating to heart failure and death of cardiac myocytes. (1) We have investigated a changed intramitochondrial free Ca^{2+} ($[Ca^{2+}]_m$) as a possible mechanism of the less active pyruvate dehydrogenase which we identified in 2 strains of hamster with an inborn cardiomyopathy (B10 14.6 and TO.2) and described in last year's report. Work with isolated mitochondria showed similar gradients of Ca^{2+} across the mitochondrial membrane in myopathic and healthy strains - i.e. no intrinsic change in the mitochondrial Ca^{2+}-transport proteins per se. However, measurements of $[Ca^{2+}]_m$ in situ in isolated cardiac myocytes from myopathic animals showed lower values compared to healthy animals, thought to be due to lower transients in cytosol $[Ca^{2+}]$ on electrical excitation. These results provide a possible mechanism for the impaired dehydrogenase regulation in this animal. (2) Mitochondrial function has been evaluated in isolated cardiac myocytes using the newly-described fluorescent dye JC-1, which reports on mitochondrial membrane potential ($\Delta\psi$). De-energization of the cells due to anoxia or respiratory chain inhibition allows some remaining measure of contractile function and preservation of $\Delta\psi$. Additional inhibition of glycolysis leads to collapse of both parameters. Evidently ATP derived from glycolysis can sustain $\Delta\psi$, under the experimental conditions employed, through a reversal of the mitochondrial protontranslocating ATPase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00243-06 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Augmented Spontanenous Ca^{2+} Release in Senescent

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: E. Lakatta Chilef LCS, NIA

Others: H. Spurgeon LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Characteristic changes in excitation-contraction mechanisms accompany cardiac cellular hypertrophy in response to chronic arterial pressure loading in rodents. These changes: prolongation of the transmembrane action potential and cytosolic calcium transient following excitation; reduction of the velocity of Ca^{2+} uptake into sarcoplasmic reticulum may be considered adaptive. They permit an extended duration of load bearing capacity following excitation. However, under conditions of a Ca^{2+} stress, e.g. following rapid pacing or elevated bathing $[Ca^{2+}](Ca_o)$, there is an increased likelihood for cellular Ca^{2+} overload to occur. Myocardial cellular hypertrophy and a pattern of excitation-contraction adaptations identical to pressure overload in younger rodents occur with advanced age in normotensive rodents. The present study examines whether, as in the pressure hypertrophied myocardium, an occult form of Ca^{2+} intolerance exists in aged myocardium. Following the imposition of Ca^{2+} stress, the manifestations of Ca^{2+} overload are more likely to occur in older vs younger heart. To determine whether myocardial Ca^{2+} intolerance, manifest as Ca^{2+} dependence after contractions (AC) or ventricular fibrillation (VF) differ in advanced age, isolated, isovolumic AV-blocked hearts from 24-26 mo (O) and 5-7 mo male rats (Y) (n=8 each) were perfused at constant flow (HEPES, 37°C) with $[Ca^{2+}]$ varied from 1.5 to 10 mM. The $[Ca^{2+}]$ required for both AC, measured from pressure recording following cessation of pacing at 2.0 Hz, and for VF, recorded via surface electrogram and occurring spontaneously during pacing, is reduced in O. VF occurred in no Y but in 6 of 8 O hearts. The results indicate an enhanced likelihood for occurrence of Ca^{2+} dependent abnormalities of cardiac cell function occurs with adult aging. The senescent (24-26 mo) rat heart exhibits exaggerated myocardial and cellular Ca^{2+} intolerance compared to its younger adult counterpart. Ca^{2+} intolerance is manifest as a lesser increase in systolic pressure coupled with an exaggerated increase in resting pressure and a greater likelihood for aftercontractions and VF to occur as perfusate [Ca] is increased. Spontaneous Ca^{2+} oscillations observed directly in cells, or manifest indirectly as SLIF in the perfused heart are the cellular basis for the Ca^{2+} dependent increase in resting pressure, aftercontractions and possibly for VF.

Discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00249-06 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Subcellular Calcium Ion Homeostasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R. G. Hansford	Chief, EMBS	LCS, NIA
Others:	B. Hogue	Chemist	LCS, NIA
	F. Di Lisa	Visiting Associate	LCS, NIA

COOPERATING UNITS (if any)

Cardiac Function Section, LCS; Department of Anesthesiology, Johns Hopkins University (Dr. D. W. Wheeler)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the mechanism whereby cells achieve homeostasis of Ca^{2+} ion concentration, both within the cytosol and other cellular compartments, and allow changes in Ca^{2+} in response of hormones and neurotransmitters. This year we have studied the response of intramitochondrial free Ca^{2+} ($[Ca^{2+}]_m$) to stimulation of isolated, single rat cardiac myocytes, both electrically and by β-adrenergic agonists. Increasing frequency of electrical stimulation, over the range 0 to 2 Hz, caused modest increases in $[Ca^{2+}]_m$: β-adrenergic stimulation had no effect on the quiescent cell (mean $[Ca^{2+}]_m$ = 75 nM) but caused a marked increase in cells stimulated at 2 Hz (mean $[Ca^{2+}]_m$ increased from 120 to 570 nM). Further, when $[Ca^{2+}]_m$ was raised in non-electrically stimulated cells, e.g. by partial Na^+-replacement, β-adrenergic stimulation had no effect. Thus, the effect of adrenergic agonists on $[Ca^{2+}]_m$ is via changes in systolic transients in Ca^{2+}, rather than on the mitochondrial Ca^{2+} transport proteins *per se*. Values of $[Ca^{2+}]_m$ in cells stimulated at near-physiological frequencies, viz 4 Hz, are in the range (~600 nM) giving approximately 50% stimulation of mitochondrial dehydrogenases. This project is a collaboration with the Cardiac Function Section, LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00259-04 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Signal Transduction of Opioid Receptor Stimulation of Myocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E. Lakatta	Chief	LCS, NIA
Others:	R-P. Xiao	Visiting Associate	LCS, NIA
	H. Spurgeon	Research Physiologist	LCS, NIA
	M. Capogrossi	Medical Officer	LCS, NIA

COOPERATING UNITS (if any)

Department of Biochemistry, University of Bologna Medical School, Bologna, Italy.

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The recent discovery of the presence of opioid peptide receptors on cardiac ventricular cells has prompted investigation of functional effects that result from stimulation of these receptors. In this regard, a naturally occurring opioid peptide, Leucine enkephalin (Leuenk), a δ receptor agonist, leads to a marked reduction in the twitch amplitude of single adult rat ventricular myocytes. This effect is due, in large part, to a reduction in the amplitude of the cytosolic Ca^{2+} transient (Ca_i). The Ca_i transient following the excitation of heart cells is due to activation of L-type sarcolemmal Ca^{2+} channels leading to Ca^{2+} influx via these channels (I_{Ca}). This Ca^{2+} influx triggers Ca^{2+} release from the sarcoplasm reticulum (SR) and also loads the SR with Ca^{2+} for subsequent releases. The specific mechanism by which Ca_i is reduced by Leuenk have been partly elucidated. Leuenk causes a release of Ca^{2+i} from the SR and leads to a reduction in the amount of Ca^{2+} in the SR stores. These effects may be attributable to an increase in IP_3 and IP_4 produced by Leuenk. However, it is unknown whether Leuenk also decreases I_{Ca}. In this regard, the effect of opioid peptides to block neurotransmitters release from neurons has been attributed to a reduction in Ca^{2+} channel current in these cells. In the present study we determined the effect of Leuenk on I_{Ca} of individual cardiac ventricular cells freshly isolated from adult rats. I_{Ca} was measured via patch pipette in the whole cell voltage clamp mode. We observed that Leuenk (10^{-7}M) decreases the amplitude of I_{Ca} by 40% during regular stimulation at 0.2 Hz at 23°C. Thus the I_{Ca} IV relation was not altered by Leuenk. The I_{Ca} depression by Leuenk was abolished by Naloxone, a specific δ receptor antagonist. The I_{Ca} inactivation kinetics were unaffected by Leuenk. That Leuenk decreases the magnitude of I_{Ca} indicates that stimulation of δ opioid receptors leads to both a reduction in the magnitude of the trigger for Ca^{2+} release and to a reduction in Ca^{2+} loading of the SR. Thus, the opioid peptide effects to decrease the Ca_i transient and contraction amplitudes in individual cardiac ventricular cells, are, in part, due to a reduction in I_{Ca}.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00261-04 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Secondary $[Ca^{2+}]_i$-Dependent Modulation of Contractility in Single Cardiac Myocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	S. J. Sollott	Senior Staff Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is well established that the primary modes of altering myocardial contractility on a beat-to-beat basis involve both the modulation of the myoplasmic Ca^{2+} ($[Ca^{2+}]_i$) availability at the level of the myofilaments (MF), and changes in the Ca^{2+}-response of the MF, especially via length-dependent activation. Alternatively, certain more slowly developing covalent modifications of MF have been recognized (such as thick or thin filament phosphorylation)., but these are of uncertain functional significance in cardiac tissue. Hypothesizing that the $[Ca^{2+}]_i$ "history" regulates contractility via such covalent modification, we performed $[Ca^{2+}]_i$-clamp experiments at various levels (ranging up to 10-fold > resting $[Ca^{2+}]_i$) and durations (up to 60s) via 10 hz tetanization in sarcoplasmic reticulum-disabled (thapsigargin or caffeine treated) intact rat cardiac myocytes loaded with indo-1 free acid. We observed that the rising phase of the tetanus (2-3 sec) was sufficiently slow such that Ca^{2+}-MF binding, and thus force and cell length, were throughout at equilibrium, enabling a rapid assessment of baseline contractile activation. A secondary $[Ca^{2+}]_i$- and time-dependent increase in cell shortening was observed despite steady levels of clamped $[Ca^{2+}]_i$, and without significant change in pH_i, Mg_i or ATP_i (as assessed by SNARF and mag-indo-1 loaded cells). This secondary leftward shift in the length-pCa curve was attenuated by the calmodulin inhibitors chlorpromazine, W7, and Sr^{2+}, and by the myosin light chain kinase (MLCK) inhibitor KT5926, and was amplified by the phosphoprotein phosphatase inhibitor Calyculin A. We propose that $[Ca^{2+}]_i$ history, via Ca^{2+}-calmodulin-dependent MLCK activity, myosin light chain phosphorylation, and kinase/phosphatase balance, plays a significant modulatory role in the chronic regulation of contractility in intact heart cells. This integrative phenomenon would reflect a history dependence of cardiac work/demand, and may serve as an important cardiovascular adaptive mechanism in myocardial conditioning.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00263-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism Affects Calcium in Cultured Aortic Endothelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	H. Silverman	Guest Researcher	LCS, NIA
Others:	R. Ziegelstein	Clinical Associate	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
	M. Stern	Guest Researcher	LCS, NIA
	E. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

Johns Hopkins University, Division of Cardiology (H. Silverman, M. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8?	0.6?	0.2?

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hypoxia is known to affect both endothelial cell adenine nucleotide metabolism and release of vasoactive substances, but the link between these is unknown. Since the release of these vasoactive substances may be in part mediated by an increase in $[Ca^{2+}]_i$ we investigated the effects of metabolic inhibitors on $[Ca^{2+}]_i$ in indo-1 loaded cultured rat aortic endothelial cells. Inhibition of oxidative phosphorylation (NaCN, 2 mM) or substrate deprivation alone (no glucose) caused little change in $[Ca^{2+}]_i$. In contrast, combined inhibition of oxidative phosphorylation and substrate deprivation (NaCN, no glucose) caused a significant increase in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ rose similarly even when cells were studied in the absence of external Ca^{2+}. The greatest increase in $[Ca^{2+}]_i$ occurred in the absence of substrate and during exposure to an inhibitor of glycolysis (iodoacetate, 2 mM). These results suggest a lack of endogenous oxidative substrate and a critical dependence on glycolytic metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00264-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Left Ventricular Volumes at Rest and During Stress in Healthy Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E. Lakatta	Chief	LCS, NIA
Others:	J. Fleg	Staff Cardiologist	LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Johns Hopkins Hospital (D. Renlund, S. Schulman, G. Gerstenblith, L. Becker)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Radionuclide ventriculography can accurately track changes in left ventricular (LV) volumes at rest and during vigorous aerobic exercise (EX). Although age-associated changes in LV volumes and ejection fraction (EF) have been shown during EX in prior studies, the role of age changes in cardiovascular fitness on these LV parameters during EX has not been assessed. To determine the effect of age on EX LV volumes, independent of physical fitness level, we obtained gated cardiac blood pool scans during graded maximal upright cycle EX in 95 healthy men ages 23-86 yr. At maximal effort in the overall group, LV end-diastolic volume index (EDVI) and end-systolic volume index (ESVI) increased with age ($r = 0.37$, $p<.01$ and $r = 0.46$, $p<.001$ respectively) while LVEF declined with age, $r = -0.46$, $p<.001$ and stroke volume index (SVI) was unrelated to age. In a subset of 49 men of homogeneous fitness (maximal workload 125-150 watts), EDVI and ESVI increased and LVEF decreased with age as in the overall sample, but SVI increased with age ($r = 0.39$, $p<.01$) due to an accentuated increase in EDVI ($r = 0.51$, $p<.001$). Age differences in the cardiac response to beta-adrenergic stimulation may underlie aging changes in cardiovascular EX hemodynamics. One way to test this hypothesis is to administer a beta-adrenergic blocking drug to normal individuals across a broad age range and compare the EX response to that in the unblocked condition The response to strenuous aerobic EX is mediated in large part by beta-adrenergic activation, the efficiency of which declines with advancing age. To ascertain whether acute beta-blockade would attenuate these normative age changes in cardiovascular EX performance, we performed maximal cycle EX in a group of BLSA men after intravenous propranolol. In 25 men ages 28-72 yr given intravenous propranolol, EDVI at peak workload declined with age ($r= -0.20$) causing an age-associated decline in SVI ($r= 0.48$, $p<0.05$) not present in the unblocked men. The decline in heart with age in propranolol-treated men was blunted (0.46 beats/min/yr) compared to controls (1.09 beats/min/yr). Max-imal LVEF declined with age similarly in the presence ($r= -0.50$, $p<.01$) and the absence ($r= -0.45$, $p<.0001$) of beta-adrenergic blockade. The primary reason for the slope shifts in the age regressions in the presence of propranolol was a large increase in EDVI and SVI and a large decrease in HR in younger men. Thus, while the age-associated decline in maximal LVEF is unaffected, acute beta-blockade reverses the age-associated ventricular dilatation during upright cycle and blunts the de-cline in maximal HR. The net effect is an accentuated decline of maximal CI with age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00265-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Age and Gender on Cardiac Structure and Exercise Cardiac Performance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. Fleg	Staff Cardiologist	LCS, NIA
Others:	F. O'Connor	Chemist	LCS, NIA
	E. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Johns Hopkins Hospital (G. Gerstenblith, J. Lima, S. Schulman, L. Becker, V. Coombs, J. Clulow).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	1.5	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To determine the independent effects of age and gender on the left ventricular structure and response to upright cycle exercise, we analyzed resting echocardiograms and gated blood pool scans at rest and maximal upright cycle exercise in 95 men and 50 women ages 23 - 86 yrs free of heart disease by history, physical exam, rest and exercise ECG and if > 40 yr old, exercise thallium scan. Left ventricular end-diastolic wall thickness increased with age (r = 0.42) and with body surface area (r = 0.38) in both sexes; after normalization for body size, there was no gender difference in the age-wall thickness relationship. Maximal cycle workload declined similarly with age in men (41%) and women (47%) between the third and ninth decades, although the absolute maximal load achieved was higher in men for any given age. At seated rest age-associated declines in heart rate (HR) and increases in systolic blood pressure (SBP) were observed in both sexes. Resting end diastolic volume index (EDVI) and stroke volume index (SVI) rose with age in men but not in women. At a fixed external workload of 50 watts, heart rate decreased and EDVI, ESVI, and SVI increased with age in men but not in women. In both sexes, maximal heart rate, ejection fraction and cardiac index declined with age whereas end systolic volume index (ESVI) and total systemic vascular resistance (TSVR) increased. Although EDVI at maximal effort increased with age in men but not in women, SVI was not age related in either sex. When men and women younger than 40 years who achieved similar maximal workloads of 125-150 watts were compared across workloads, women had higher heart rates and thence higher cardiac indices than their male counterparts whereas the men had higher systolic blood pressure responses. Fitness-matched older (> 60 years) men and women demonstrated similar exercise hemodynamics except for a higher heart rate response in the latter. Thus, gender has a significant influence on the age-associated changes in resting and exercise hemodynamics in normal humans. Older men appear to utilize the Frank-Starling mechanism to augment cardiac output more than older women, whereas the latter have a higher heart rate response. However, the apparent gender differences in left ventricular wall thickness are eliminated by normalization for body size.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00266-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Transport Mechanisms and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. P. Froehlich	Chief, MBS	LCS, NIA
Others:	J. L. Kinsella	Research Physiologist	LCS, NIA
	P. F. Heller	Chemist	LCS, NIA
	R. W. Albers	Chief, Enzymes Section	LNC, NINCDS

COOPERATING UNITS (if any)

Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD; Max-Planck-Institute for Biophysics, Frankfurt, Germany (K. Fendler); Dept. Biochemistry, U. of Minnesota (D. Thomas and J. Mahaney).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Both Na,K-ATPase and Ca-ATPase are critical cation transport systems in membranes of eucaryotic cells. The plasma membrane Na,K-ATPase pumps Na^+ out of cells in exchange for K^+. Sarcoplasmic reticulum Ca-ATPase is important for lowering intracellular Ca^{2+} in the heart between contractions. Increasing our insight into the mechanism and the regulation of these enzymes is of widespread interest at the molecular, the membrane, the cellular, and the whole animal level. The most fundamental questions involve 1) the free energy coupling between ATP hydrolysis and ion transport, 2) the molecular structure of the ion channels, and 3) the regulation of enzyme activity to meet the needs of the cell and the organism. Insight into these mechanisms may be crucial for our understanding of disease and aging.

In a series of experiments, we have gained insight into the nature and mechanism of the three Na^+ binding sites within the ion channel of the Na,K-ATPase. A model for Na^+ transport by electric organ Na,K-ATPase is proposed based on the statistical partitioning of Na^+ binding states. The model accounts for (1) the biphasic time course of phosphorylation obtained under conditions where Na^+ is added last, and (2) the conversion to monophasic kinetics when the enzyme is preincubated with Na^+ prior to ATP addition. These results imply that the third Na^+ binding site is restricted when the other two sites are occupied.

In other experiments we examined the coupling of Ca transport to ATP hydrolysis in isolated cardiac sarcoplasmic reticulum. Quenched-flow measurements of phosphoenzyme formation by the Ca^{2+} pump in sarcoplasmic reticulum (SR) were performed at 2°C for comparison with EPR (electron spin resonance) spectral data demonstrating a slow conformational change associated with active Ca^{2+} transport. Phosphoenzyme formation was biphasic with a close correlation between the slow phase of phosphorylation and the EPR spectral change. The results indicate that the spin label senses a protein conformational change linked to phosphorylation of the Ca^{2+} ATPase or to the interconversion of subsequently-generated phosphorylated intermediate states. These studies provide important information about the mechanism for free energy coupling between ATP hydrolysis and Ca^{2+} transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00267-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Cell Function and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	L.L. Cheng	Research Chemist	LCS, NIA
	J.P. Froehlich	Chief, MBS	LCS, NIA
Others:	E. Koh	Visiting Fellow	LCS, NIA
	E.G. Lakatta	Chief, LCS	LCS, NIA
	J.L. Kinsella	Research Physiologist	LCS, NIA
	C.T. Liang	Research Chemist	LBC, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry, NIA (C.T. Liang)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.45	2.45	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Aging constitutes a risk factor in the development of cardiovascular disease. Older animals are more susceptible to vascular lesions after endothelial injury than are young animals. The mechanism leading to the increased proliferation of SMC in the intima is unknown. However, the intimal thickening may result from excessive production of growth factors and/or increasing the activities of proteinases known to enhance the degradation of the extracellular matrix components. The purpose of these studies was to examine the factors involved in the development of intimal thickening. Histological staining of the thoracic aorta with TGF-ß specific antibodies have revealed the presence of higher levels of extracellular $TGF\beta_1$ in the basement membranes from old rats as compared to young. In contrast, no apparent age-related differences were detected in the levels of intracellular $TGF\beta_1$ or extracellular $TGF\beta_2$ and $TGF\beta_3$. Significant elevations in collagenase activity were detected in aortic homogenates from old and balloon injured animals. These elevations may be involved in the degradation of matrix protein associated with SMC migration and intimal thickening.

B. Age-related alterations in smooth muscle ion metabolism may contribute to the onset and progression of hypertension. A model for the investigation of this process involves freshly-isolated single rat arterial smooth muscle cells (SMC). These cells were used to investigate the relationship between intracellular $[Ca^{2+}]$ fluctuations and initial velocity of cell shortening induced by norepinephrine and K^+ depolarization. The results showed that single cells retain a Ca^{2+}-dependent variation in shortening velocity consistent with the behavior observed in multicellular (tissue) preparations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00268-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cardiac Gene Expression With Adult Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E.G. Lakatta	Chief	LCS, NIA
Others:	L. O'Neill	Chemist	LCS, NIA
	N.J. Holbrook	Chief, UEA	LMG, NIA
	J. Fargnoli	Senior Staff Fellow	LMG, NIA

COOPERATING UNITS (if any)

INESRM U 127, Hôpital Lariboisière, Paris, France (A. Lompré, K. Schwartz, K. Boheler, L. Carrier, C. Wisnewsky, C. Chassagne, D. de la Bastie)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cardiac muscle of senescent rodent hearts exhibits cardiac cell enlargement and a pattern of altered cardiac excitation-contraction mechanisms, among which are a reduced contraction velocity, a reduction in the myosin ATPase activity, a prolonged contraction time and a reduced rate of Ca^{2+} sequestration into isolated sarcoplasmic reticulum (SR). The present study was undertaken to determine whether the age-associated phenotypic changes are also associated with changes in myosin heavy chain (MHC) or SR Ca ATPase gene expression. Levels of mRNA coding for α and β MHC, SR Ca ATPase, calsequestrin, an SR Ca^{2+} binding protein, and the α-skeletal and cardiac isoforms of actin were determined by Northern analysis and dot blots on RNA purified from 6 hearts each, of animals of a broad age range. The mRNA for β MHC increased greater than fourfold from 1 to 24 months, the α mRNA for α MHC decreased by a proportional amount. The mRNA for SR Ca ATPase decreased by 50% from 12-24 mo. Neither calsequestrin nor α-skeletal or cardiac actin isoforms changed with adult age. Additionally, S_1 nuclease mapping analysis revealed that neither the Ca^{2+}-ATPase nor the calsequestrin gene products expressed in heart were differentially spliced during aging. Thus, the phenotypic, biophysical and biochemical cardiac contractile changes with adult aging are, in part at least, transcriptionally regulated: the MHC isogene shift produces different isoforms of MHC proteins. Actin isogenes do not differentially express their products with adult aging. While the level of the SR mRNA declines with senescence there is no evidence for an isoform shift. The Ca binding protein gene, calsequestrin does not appear to change its expression or isoform with aging.

Combined into Z01 AG 00804-01 LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00269-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

α-Adrenergic Stimulation in Myocardial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	G. Gambassi	Visiting Fellow	LCS, NIA
Others:	H. A. Spurgeon	Physiologist	LCS, NIA
	E. G. Lakatta	Chief	LCS, NIA
	P. S. Blank	IPA	LCS, NIA
	B. D. Ziman	Guest Researcher	LCS, NIA
	M. C. Capogrossi	Medical Officer	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Different aspects of myocardial α_1-adrenoceptor (α_1-AR) stimulation were examined: 1) The positive inotropic action of α_1-adrenoceptor stimulation, at least in part is due to an enhanced myofilament responsiveness to Ca^{2+} mediated by activation of Na^+/H^+ exchange and an increase in cytosolic pH (pH_i). We tested the hypothesis that the increase in pH_i may be due to protein kinase C-mediated activation of Na^+/H^+ exchange. In experiments with isolated rat myocytes α_1-AR stimulation with phenylephrine and nadolol enhanced contraction and pH_i. However, following PKC inhibition with staurosporine or downregulation with overnight exposure to a tumor-promoting phorbol ester, α_1-AR stimulation had no effect on pH_i and decreased twitch amplitude. Thus, PKC-mediated activation of Na^+/H^+ exchange increases pH_i and contributes to the positive inotropic action of α_1-AR stimulation. In contrast, inhibition of PKC-dependent effects makes apparent a negative inotropic effect of α_1-AR stimulation. 2) We examined the effect of α_1-AR subtypes, α_{1A} and α_{1B} on contraction, Ca_i and myofilament response to Ca^{2+} of isolated myocardial cells. α_{1A}-AR stimulation (phenylephrine, nadolol and α_{1B}-AR inactivation with chloroethylclonidine) increased contraction, Ca_i transient amplitude and myofilament response to Ca^{2+}. In contrast α_{1B}-AR stimulation (phenylephrine, nadolol and α_{1A}-blockade with WB-4101) decreased contraction, Ca_i transient amplitude and downregulated the α_{1A}-AR effect to increase myofilament response to Ca^{2+}. 3) Myocardial damage and arrhythmias due to reperfusion after ischemia are worsened by α_1-AR stimulation and improved by acidosis. We examined the effect of α_1-AR on pH_i and aftercontractions during recovery from hypercarbic acidosis. α_1-AR increased pH_i and the frequency of aftercontractions upon recovery from acidosis. Both effects were prevented by ethylisopropylamiloride (EIPA), a blocker of Na^+/H^+ exchange. Under similar experimental conditions α_{1A}-AR stimulation enhanced and α_{1B}-AR stimulation markedly decreased the frequency of aftercontractions over the effect on non-selective α_1-AR stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00270-03 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Changes in Vascular Stiffness Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E. Lakatta	Chief	LCS, NIA
Others:	P. Vaitkevicius	Guest Researcher	LCS, NIA
	J. Fleg	Staff Cardiologist	LCS, NIA
	J. Engel	Computer Scientist	LCS, NIA
	H. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

University of Maryland (A. Goldberg, L. Lakatta)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As arterial and myocardial changes occur in otherwise healthy older individuals within the Western society they are often referred to as a part of a "normal aging process." However, the arterial stiffness increase (indexed by an increase in arterial pulse wave velocity) and the arterial pressure increase with age are heterogeneous among various populations worldwide and among individuals within a given population. The ultimate goals of this project are to address the issue of how alterations in arterial stiffness affect the myocardium and whether in outcome studies they relate to vascular insufficiency syndromes, e.g., stroke. We have initiated a pilot study in which measurements of cardiac mass (via NMR), filling properties and isovolumic relaxation time (via Doppler echocardiography), carotid pressure pulse (via applanation tomography) and arterial pulse wave velocity (Doppler sonography) are made in men and women who differ with respect to age, arterial pressure, body composition, and physical conditioning status. As expected, results indicate that with increasing age arterial stiffening leads to an increase in pulse wave velocity. This is associated with an early return of reflected pulse waves from peripheral sites which produce an augmented and late occurring peak of carotid pressure pulse. It is important to note that these arterial changes are, by and large, not detected by routine clinical measures of brachial arterial pressure and are independent of gender, but vary inversely with exercise capacity. Of note, also is that in highly physically conditioned older individuals (>60 yrs of age) the arterial stiffness and reflected wave indices are markedly reduced and do not differ from those of younger individuals, although the age-associated increase in systolic pressure persists. We have taken the initiative to extend many of these studies to Black Americans and to other non-Western populations in which different patterns of arterial pressure change occurs with aging (in China via a research contract).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00271-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Cardiac Opioids in Response of the Heart to Stress

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Boluyt	NRC Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	L. O'Neill	Biologist	LCS, NIA

COOPERATING UNITS (if any)

Department de Biologie Appliquee, Universite d'Auvergne Clermont, Aubiere, France (A. Younes); Texas College of Osteopathic Medicine, Fort Worth, Texas, (J. Caffrey)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The magnitude of cardiac hypertrophy is altered with advancing age and is accompanied by an increase in cardiac opioid peptide production. Previous work in the laboratory has shown that opioid peptides can directly modulate cardiac myocyte contractile function and inhibit catecholamine induced inotropy by affecting signal transduction through the ß-adrenergic pathway. Since cardiac hypertrophy is believed to be a regulated in part by the ß-adrenergic signal transduction pathway, we hypothesized that cardiac opioids regulate the hypertrophic process. The objectives of this study were to determine whether the cardiac response to hypertrophic stress is associated with changes in opioid mRNA and peptide levels in rat heart, and whether opioid gene expression in the heart is mediated by adrenergic stimulation. Cardiac opioid production was upregulated 2-3-fold with advancing age, and proenkephalin (PNK) gene expression was elevated in the heart during aging (7-fold), and during chronic pressure overload hypertrophy leading to heart failure (2-fold). Paradoxically, PNK gene expression is transiently downregulated after 3 days of aortic constriction, and this transient decrease was abolished by α-adrenergic blockade. Results of studies on cultured neonatal cardiac myocytes indicate that: 1) PNK mRNA abundance is increased in myocyte cultures supplemented with norepinephrine (NE), peaking at 4 hours after addition, 2) α- and ß-adrenergic receptor stimulation act synergistically to increase the expression of PNK, and 3) myocyte cultures of high purity permit the induction of hypertrophy marker genes but not PNK by NE treatment, while the opposite is true for cultures containing cardiac fibroblasts. The mutually exclusive expression of PNK and markers of hypertrophy suggests that opioid peptides may inhibit cardiac myocyte hypertrophy. In addition, the level of PNK induction achieved in mixed cultures by NE treatment is sufficiently higher than that in cultures of cardiac fibroblasts, suggesting that a myocyte-fibroblast interaction exists which enhances the PNK expression in one or both of these cell types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00272-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms for Signal Transduction of Shear Stress Forces in Endothelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M.C. Capogrossi	Medical Officer	LCS, NIA
Others:	R.C. Ziegelstein	Clinical Associate	LCS, NIA
	L.L. Cheng	Research Chemist, MBS	LCS, NIA
	H.A. Spurgeon	Physiologist	LCS, NIA
	P.S. Blank	IPA	LCS, NIA
	E. Koh	Visiting Fellow, MBS	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry, NIA (Drs. Roberto Pili and Antonino Passaniti); Cardiology Division, Johns Hopkins University (Dr. Jay Zweier and David Lefer).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Wall shear stress in blood vessels plays a role in the development of restenosis after angioplasty, coronary artery bypass graft occlusion and atherosclerosis. In addition these processes are also modulated by the interaction of vascular cells with the endothelium. The purpose of this project is: 1) To characterize, in endothelial cells (EC), the effect of acute changes in shear stress forces on cytosolic pH $(pH)_i$ and $[Ca^{2+}](Ca_i)$. 2) To determine the effects of changes in Ca_i on EC's pH_i and 3) to determine how the interaction between the endothelium and other cells affects Ca_i homeostasis in EC. The effect of laminar shear stress on EC's pH_i was studied by examining EC cultured in glass capillary tubes. Shear stress forces led to a rapid decrease in EC's pH_i in the presence of HCO_3^-. This response was markedly blunted by the anion exchange inhibitor 4-acetamido,4'-isothio-cyanatostilbene-2,2'-disulfonic acid (SITS) but unaffected by Na^+ removal. In the absence of HCO_3^-, shear stress forces caused a small increase in pH_i which was abolished by ethylisopropylamiloride (EIPA), a Na^+/H^+ exchange inhibitor. The effect of acidification on Ca_i was examined in the absence of significant shear stress forces either by removal of NH_4Cl, changing from a bicarbonate-free to a 5% CO_2/HCO_3^--buffered solution at constant buffer pH, or changing from a 5% CO_2/HCO_3^- to a 20% CO_2/HCO_3^- solution. Regardless of the method employed, intracellular acidification resulted in an increase in Ca_i indexed by the fluorescent Ca^{2+} indicator indo-1. The increase in the indo-1 fluorescence ratio induced by changing from a 5% CO_2/HCO_3^- to a 20% CO_2/HCO_3^- solution was not significantly altered by removal of buffer Ca^{2+} either before or after depletion of bradykinin-sensitive intracellular Ca^{2+} stores. In other experiments we examined the effect of leukocyte adhesion on EC's Ca_i. Upon contact between granulocytes or monocytes with EC there was a rapid increase in EC's Ca_i which exhibited a partial recovery toward control. This response was abolished after depletion of EC's endoplasmic reticulum Ca^{2+} with thapsigargin while it was not affected in a Ca^{2+}-free buffer. Similar results were obtained when melanoma cells were used in place of leukocytes. EC contact with 8 μm inert beads did not elicit an increase in Ca_i.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00273-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MyoD1 and Developmental Gene Expression in Skeletal Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Crow	Senior Staff Fellow	LCS, NIA
Others:	N. Papadopoulos	Visiting Fellow	LCS, NIA

COOPERATING UNITS (if any)

Baylor College of Medicine, Houston, Texas (Robert Schwartz); University of Texas, Houston, Texas (Eric N. Olson)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The skeletal muscle regulatory factors (MRFs), of which myoD is the prototype, are a family of helix-loop-helix proteins that act as transcription factors for skeletal muscle-specific gene expression. The various members of the MRF family are differentially expressed during development suggesting that they may participate in orchestrating developmental changes in skeletal muscle maturation. To address this, we have focussed on the changes in MRF gene expression that occur between embryonic and fetal stages of chicken skeletal muscle development. This transition involves the switching of contractile protein isoform gene expression from the "cardiac" to the "skeletal" type, occurs in concert with increases in muscle activity, and is associated with increased protein kinase C (PKC) activity. In work completed this year, we show that expression of the MRFs and "cardiac" type contractile proteins mRNA in cultured muscle cells are decreased when PKC levels are elevated either pharmacologically or genetically. We also show that the effect of PKC on myoD is at the level of gene transcription, but cannot be overcome by overexpression of myoD driven by a heterologous promoter. Since MRFs are likely to positively regulate their own gene expression, the data is consistent with a model of regulation in which the primary effect of PKC is post-transcriptional, creating a defunct transcription factor that can neither transactivate its own gene or that of its targets. Additional information suggests that the effect is likely mediated by direct phosphorylation of a threonine residue in the DNA binding domain of the MRFs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00274-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Smooth Muscle Gene Expression and Cellular Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Crow	Senior Staff Fellow	LCS, NIA
Others:	E. Lakatta	Chief	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
	R. Pauly	Medical Staff Fellow	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	N. Papadopoulos	Visiting Fellow	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The phenotypic modulation of vascular smooth muscle cells (VSMCs) from a quiescent, differentiated state to that of proliferating, "undifferentiated" state is a common pathogenic feature of vascular disease. We had previously shown that proliferating VSMCs express a mRNA with strong homology to the mouse ID (Inhibitor of Differentiation) gene and this expression was downregulated when these cells differentiated. In skeletal muscle, the product of the ID gene is thought to acta as a transdominant suppressor of the muscle regulatory/transcription factors that belong to the helix-loop-helix family of proteins. Expression of ID in proliferating skeletal muscle precursor cells and its downregulation preceding differentiation prevents HLH muscle transcription factors from activating skeletal muscle-specific gene expression in the proliferating precursor cells. Using a variety of molecular cloning approaches, we have identified and cloned the cDNAs for three putative ID-like proteins that are expressed in proliferating VSMCs. We have identified one of these as rat ID, the rate homologue of the original mouse ID cDNA. Rat ID is expressed at low levels in the vessels of mature rats, but at very high levels in proliferating VSMCs derived from these vessels. Its expression is significantly reduced by conditions that bring about growth arrest of the cells, such as confluence, serum withdrawal, or contact with basement membrane matrices. VSMC cultures established from the vessels of older rats also express high levels of rat ID as they proliferate, but unlike "younger" VSMCs do not downregulate ID levels in response to those manipulations that lead to growth arrest in the "younger" cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00275-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of Myofilament Ca^{2+} Sensitivity as a Positive Inotropic Intervention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E.G. Lakatta	Chief	LCS, NIA
Others:	G. Gambassi	Visiting Fellow	LCS, NIA
	M.C. Capogrossi	Medical Officer	LCS, NIA
	H.A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

E. Merck, Darmstadt, Germany (M. Klockow)
Dept. of Physiology, University of Chicago at Illinois (J.R. Solaro)
Dept. of Physiology, University of Vermont (D. Warshaw)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A persistent challenge has been the development of substances that increase myocardial contractility via an enhancement of myofilament responsiveness to Ca^{2+} rather than by increasing the extent of cellular Ca^{2+} loading. The diazinone derivative, EMD 53998 (designed by E. Merck, Darmstadt, Germany), increases the peak force and shift leftward the pCa-force relationship in skinned myocardial fibers; and in cell homogenates it exhibits phosphodiesterase (PDE) inhibitory activity. However, the potency of myofilament sensitization relative to that of PDE inhibition is greater than for any known substance. The effects of EMD 53998, and of its (+), EMD 57033 and (-), EMD 57439, enantiomers, were tested on the contractile properties and Ca_i transients of single, intact, guinea pig and dog cardiac myocytes. Cells were loaded with the fluorescent dye, indo-1, and bathed in a Hepes buffer at 25°C. Our aim was to ascertain whether the optical enantiomers could separate the effect mediated through PDE inhibition from that obtained via an increased myofilament responsiveness to Ca^{2+}. All three substances exerted a pronounced increase in twitch amplitude: the maximal effect of the racemate was approximately the sum of the effects of its two enantiomers. The Ca_i transient, measured as the 410/490 nm indo-1 fluorescence ratio transient, was increased by the racemate and its (-) enantiomer, but not by the (+)-enantiomer. In unstimulated cells resting length was significantly reduced by the (+)-enantiomer and this was accompanied by a decrease in indo-1 fluorescence; the (-)-enantiomer had no effect on either parameter. Qualitatively similar effects were obtained in experiments with intact dog cardiac cells. The molecular mechanism of the effect of the (+)-enantiomer was further studied in dog cardiac myofibrils. EMD 57033 stimulates the ATPase activity in myofibrils in which troponin-tropomyosin have been extracted, but does not affect Ca^{2+} binding to isolated troponin C. Furthermore, in a motility assay containing isolated actin and myosin, but devoid of Ca^{2+} and regulatory proteins, the substance increases the velocity of actin motion along myosin. Thus, in intact cells the (+)-enantiomer of EMD 53998 has direct myofilament effects which are not simply due to "Ca^{2+}-sensitization" (i.e. effects downstream to Ca^{2+}-binding to troponin C and regulatory protein modulation of thin filament activation); rather an effect on acto-myosin interaction, i.e. the cross-bridge is likely involved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00276-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mitochondrial and Cytosolic Calcium in Cardiac Myocyte After Anoxia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	H. Silverman	Guest Researcher	LCS, NIA
Others:	H. Miyata	Visiting Fellow (DOD 2/25/92)	LCS, NIA
	E. Lakatta	Chief	LCS, NIA
	M. Stern	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

Johns Hopkins University, Division of Cardiology (H. Silverman, M. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies from this laboratory and others have documented an association between cellular Ca^{2+} loading and cardiac myocyte death following exposure to anoxia. It remains unclear whether these changes in Ca^{2+} mediate cell injury and how this may occur. We characterized the changes in Ca^{2+} which occurred in the cytosolic compartment $[Ca^{2+}]_c$ and mitochondrial compartment $[Ca^{2+}]_m$ of adult rat cardiac myocytes exposed to hypoxia/reoxygenation to define the relation between subcellular Ca^{2+} regulation and cell injury. During anoxia, $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ rose only after the onset of ATP-depletion rigor contracture. At reoxygenation 35 minutes later, $[Ca^{2+}]_c$ fell rapidly to preanoxic levels while $[Ca^{2+}]_m$ fell more slowly. Cellular hypercontracture or death was associated with a rise in $[Ca^{2+}]_c$ or $[Ca^{2+}]_m$ to greater than 250 nM just prior to reoxygenation. Reversible relatively minor rises in $[Ca^{2+}]_m$ may be seen and do not predict cell death. The cause of cell death remains unclear but does not appear to be the result of massive reoxygenation-induced Ca^{2+} overload.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00277-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Angiogenesis Endothelial Cell Function and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J. Kinsella	Research Physiologist	LCS, NIA
Others:	L. Cheng	Research Chemist	LCS, NIA
	J. Froehlich	Chief, MBS	LCS, NIA
	E. Lakatta	Chief	LCS, NIA
	M. Kuzuya	Visiting Fellow	LCS, NIA
	M. Crow	Senior Staff Fellow, CFS	LCS, NIA
	T. Passaniti	Senior Staff Fellow, RMS	LBC, NIA

COOPERATING UNITS (if any)

Laboratory of Developmental Biology, NIDR, NIH (H. Kleinman, D. Grant), University Jerusalem, Israel (R. Reich), Laboratory Clinical Physiology NIA NIH (F. Chrest).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Angiogenesis (the formation of new blood vessels) is an important process in tumor growth, wound healing, retinopathies, and rheumatoid arthritis. We are investigating aspects of angiogenesis in an in vitro model utilizing isolated human and bovine endothelial cells and a mixture of basement membrane matrix proteins (Matrigel). Plating endothelial cells on Matrigel-coated tissue culture dishes led to their rapid (12 to 18 hr) organization into a capillary-like network.

Normally endothelial cells are quiescent in vivo; but in response to an angiogenic factor, activated endothelial cells will breakdown their underlying basement membrane, migrate into the interstitium, proliferate, and finally differentiate into a new blood vessel. We found that quiescent endothelial cells (contact inhibition or serum starvation) were not able to organize into a capillary-like network in vitro, while cells released from contact inhibition or feed serum-containing media regained the ability to organize. There was a correlation between the ability to organize on Matrigel and the expression of cell surface receptors for the basement membrane proteins, laminin and collagen IV. These studies should prove useful in studying some early events involved in angiogenesis.

Often angiogenic factors attract leukocytes and fibroblast, while stimulating the formation of new blood vessels. Previous studies have demonstrated that leukocytes can release angiogenic factors, but it is unclear whether fibroblast cells may also be involved. We tested whether fibroblasts may produce factors that alter endothelial cell function. We found that fibroblast-conditioned media increased endothelial cell proliferation, contained a cellular attractant, and enhanced the extent of capillary-like formations on the Matrigel. The ability of fibroblasts to influence angiogenesis may be an important component of wound-healing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00278-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Vascular Smooth Muscle Cells in Vascular Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R. Pauly	Clinical Associate (MSF)	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	L. Cheng	Reseasrch Chemist, MBS	LCS, NIA
	S. Sollott	Senior Staff Fellow	LCS, NIA
	A. Passaniti	Senior Staff Fellow, RMS	LBC, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development and progression of several vascular diseases depend on the migration and proliferation of vascular smooth muscle cells (VSMC) and their interaction with extracellular matrix (ECM). We have found previously that this interaction is due in part to the invasion of reconstituted basement membrane (Matrigel) by VSMC in response to PDGF. Proliferative (dedifferentiated) VSMC exhibit fivefold greater invasiveness as compared with post-confluent (differentiated) VSMC. We have demonstrated that intracellular calcium dynamics play an important role in VSMC invasion but not in chemotaxis. Chemotaxis reflects the ability of cells to attach to a substrate and migrate in response to a chemoattractant. Chemoinvasion requires the additional ability of cells to degrade a barrier. We have demonstrated 72 KD type IV and 92 KD type IV collagenase activity by zymography in invasive VSMC. Northern blot analyses have indicated mRNA for 72 KD type IV collagenase but not 92 KD type IV collagenase in these cells. Invasive VSMC also express mRNA for the tissue inhibitors of matrix metalloproteinases (TIMPs) - reflecting the importance of a balance between positive and negative regulators. Chemoinvasion assays employing antiserum to the 72 KD type IV collagenase, nonimmune serum, and antiserum to the 92 KD type IV collagenase, have demonstrated that VSMC invasion in response to PDGF is mediated by the 72 KD type IV collagenase. Chemotaxis is less dependent on the 72 KD type IV collagenase. Because these collagenases are secreted in zymogen form, activation is required for degradation of ECM. Experimental results employing BAPTA (a chelator of intracellular calcium) and ionomycin (a calcium ionophore) suggest calcium may be important in this activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00279-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparison β_2 vs β_1 Adrenoceptor Stimulation in Rat Cardiocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R-P. Xiao	Visiting Associate	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	B. Tantini	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bolus β_1 and β_2 adrenoceptors exist in the heart. While the cellular effects of the former are widely studied, the specific effects of the later have not been elucidated. We studied the effects of β_2- and β_1-adrenoceptor (β_2AR and β_1AR) agonists on the cytosolic Ca^{2+} (Ca_i) transient, indexed by the transient increase in Indo-1 fluorescence ratio following excitation, twitch amplitude, measured via photodiode array, and membrane potential and L-type sarcolemmal Ca^{2+} current, and I_{Ca}, measured by whole cell patch electrode in single rat ventricular myocytes. The selective β_2AR agonist Zinterol (Zint) increased the amplitudes of both the Ca_i transient and twitch in a concentration-dependent manner. Similar results were obtained when β_2AR's were stimulated with isoproterenol (ISO) in the presence of the selective β_1AR antagonist, CGP 20712A (CGP). β_1AR stimulation induced by norepinephrine (NE) increased twitch amplitude to about the same extent as β_2AR. However, several striking differences between response to β_1AR and β_2AR stimulation were observed. β_1AR stimulation had a potent effect to abbreviate the time course of the twitch contraction and Ca_i transient; β_2AR stimulation did not reduce the time course of the Ca_i transient and had only a minor effect on the twitch duration. For a given increase in twitch amplitude, β_1AR stimulation caused a greater increase in Ca_i transient, suggesting a diminished Ca_i-myofilament interaction. β_1AR, but not β_2AR stimulation, evoked spontaneous Ca_i oscillations, increased the diastolic indo-fluorescence level and caused a decline in resting cell length. β_1AR and β_2AR also differed in their effects on I_{Ca}. While both β_1AR and β_2AR stimulation increased the peak I_{Ca} amplitude, β_2 markedly prolonged the I_{Ca} inactivation time. Accordingly, β_2AR stimulation prolonged the action potential duration to a greater extent than β_1AR stimulation did. CPT cAMP mimicked the effects of β_1AR stimulation by NE but not those due to β_2AR stimulation. These results clearly indicate that both β_2AR and β_1AR adrenoceptors functionally coexist in rat ventricular myocytes, but that stimulation of these receptor subtypes elicits qualitatively different cell responses at the levels of ionic channels, the myofilaments, and sarcoplasmic reticulum (SR).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00280-02 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interaction of σ Opioid and ß Adrenergic Receptors on Cardiac Myocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R-P. Xiao	Visiting Associate	LCS, NIA
Others:	M. Capogrossi	Medical Officer	LCS, NIA
	H. Spurgeon	Physiologist	LCS, NIA
	E. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has been recently reported from this lab that opioid receptors exist in myocardial membranes, and that opioid agonists have negative inotropic effects in the heart. In intact animals and in the isolated heart, opioid agonists inhibit NE release from neuron endings. However, whether stimulation of myocardial opioid (OP) receptors modifies the response to β-adrenergic receptor (BAR) stimulation at postsynaptic levels is unknown. Our studies in individual isolated rat ventricular myocytes (electrically stimulated at 0.5 Hz at 23°C) demonstrate that the increase in cytosolic Ca^{2+} transient (indexed by indo-1 fluorescence) and contraction (measured via photodiode array) amplitudes and increase in the sarcolemmal L type Ca^{2+} current I_{Ca}) in response to norepinephrine (NE,10^{-7}M) are abolished by the addition of 10^{-8}M Leucine enkephalin (LE), a δ OP agonist. The LE effect was reversed by Naloxone (Nal,10^{-8}M). Thus, stimulation of δ OP receptors on heart cells can "short circuit" the BAR mediated response and may protect against cell Ca^{2+} overload during stress.

Combined into Z01 AG 00259-04 LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00800-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Energy Metabolism in Aging and Disease: Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R. G. Hansford	Chief, EMBS	LCS, NIA
Others:	B. Hogue	Chemist	LCS, NIA
	I. Kudryashova	Visiting Fellow (EOD 10/91)	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise survival of neurones. We have examined the possibility that impaired mitochondrial protein synthesis may result in decreased respiratory chain function in old age. The respiratory chain complexes chosen for study are those in which one or more subunit is coded for by mT-DNA; viz, NADH-CoQ oxidoreductase (Complex 1); cytochrome c oxidase (Complex IV); F1-Fo-ATPase (Complex V). In addition, State 3 (ADP-dependent) O_2-uptake with glutamate plus malate and with succinate as substrate was studied. At this time, activities have been found to be similar in cerebral cortex, hippocampus and striatum, when young adult (6 mo) and senescent (24 mo) male rats were compared. In a collaboration with Dr. C. Filburn of LBC, we have also initiated a study of the incidence of deletions of mt-DNA in the brain regions identified above, as a function of aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00801-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Contractile Deficit of Rat Heart Cells to Norepinephrine With Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	R-P. Xiao	Clinical Associate	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	H.A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In both humans and animals the effectiveness of ß-adrenergic stimulation to augment the heart contraction strength declines with aging. We have investigated cellular mechanisms that may underlie the aging deficit. Twitch amplitude (TA), (%rest cell length), measured via photodiode array and cytosolic Ca^{2+} transient, indexed as transient in Indo-1 fluorescence ratio 410/940 μm (IF) were measured in single ventricular myocytes of rats of varying age during electrical stimulation at 23°C. The L type Ca^{2+} current (I_{Ca}, pA/pF) was measured in additional cells during voltage clamp from -40 to 0 mV for 200 msec at 0.5 Hz (CsCl, 120, ATP, 3, and EGTA 10 mM in pipette; CsCl 5, and Ca 1 mM in bath). With aging the maximum TA, IF and I_{Ca} response to NE (10^{-7}M) are blunted.

	A (n=85), each age)		IF (n=85, each age)		I_{Ca} (n=20, each age)	
	Control (C)	% C NE**	Control (C)	% C NE*	Control (C)	% C NE**
3 Mo	6.27±0.32	222.73±9.33	0.200±0.009	193.77±10.44	5.32±0.53	235.83±20.19
8 Mo	6.71±0.30	174.38±7.66	0.225 ±0.012	170.17±14.10	5.36±0.42	179.46±12.79
24 Mo	6.63±0.44	139.00±9.98	0.260±0.012	144.36±10.38	5.33±0.61	156.71±14.07

Age effect *p<0.02; **p<0.001.

Thus, the blunted Ta and CaT responses to NE with aging are due to a failure to sufficiently augment Ca^{2+} influx via I_{Ca} to trigger Ca^{2+} release from the sarcoplasmic reticulum (SR) and to Ca^{2+} load the SR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00802-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Physical Conditioning on Cardiovascular Aging Changes in Normal Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	J.L. Fleg	Staff Cardiologist	LCS, NIA
Others:	F.C. O'Connor	Chemist	LCS, NIA
	E.G. Lakatta	Chief	LCS, NIA
	A. Nussbacher	Guest Researcher	LCS, NIA
	P. Vaitkevicius	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Francis Scott Key Medical Center (E. Shapiro, J. Taube); Division of Cardiology, Johns Hopkins Hospital (G. Gerstenblith, S. Schulman, L.Becker).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because a major decline in physical conditioning status usually accompanies the aging process in industrialized societies, it is very difficult to separate the cardiovascular effects of aging per se from those of deconditioning. The following three studies seek to delineate the independent effect of these processes on left ventricular (LV) performance, at rest and during strenuous aerobic exercise. A. To determine whether intensive physical conditioning can attenuate the well-known age-associated decline in early diastolic LV function, we performed resting Doppler echocardiography on 16 competitive male endurance athletes 52-76 years, 27 age-matched sedentary men and 17 sedentary men < 40 years old, all normal by history, physical exam and maximal treadmill ECG. Although VO_{2max} in the older athletes exceeded that in both older and younger controls (47±6 vs 30±7 and 41±7 ml/kg/min, respectively), Doppler E velocity peak, E/A ratio and atrial filling fraction/AFF) resembled those of their age-peers and indicated significant impairment of early diastolic filling compared to young control subjects. These results indicate that even high levels of aerobic training and fitness do not generally offset the age-associated decline in resting early diastolic LV function. B. To determine the effect of age and conditioning status on diastolic LV performance during intense aerobic exercise, we measured radionuclide ventriculographic peak filling rates at rest and throughout graded maximal upright cycle ergometry in 56 sedentary BLSA men and 12 highly trained old men. At rest, at 50% of maximum workload and at maximal effort, peak filling rates declined with age but were similar in older athletes and their sedentary age peers. Intravenous propranolol decreased filling rates in young (37±8 yr) but not in older (62±6 yr) sedentary subjects. These results suggest that the age-associated decline in early diastolic filling is over mediated by the deficit in beta-adrenergic responsiveness seen with aging. C. To examine the cardiovascular effects of detraining in highly conditioned older men, we performed gated radionuclide ventriculography during maximal cycle ergometry in 6 senior athletes (aged 61±8 yr) before and after 12 weeks of deconditioning. Declines in VO_{2max} (51±6 to 43±7 ml/kg/min), stroke-volume index (66±13 to 54±9 ml/m^2) ejection fraction (87±5 to 80±4) and cardiac index (10.2±1.9 to 8.4±1.2 l/μm) were noted after detraining. These results suggest that detraining reduces LV systolic performance during maximal aerobic exercise in highly conditioned older men.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00803-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Patterns of Gene Expression in the Heart

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Boluyt	NRC Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	L. O'Neill	Biologist	LCS, NIA
	A. Meredith	Stay-in-Schooler	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Atrial natriuretic factor (ANF) is synthesized and secreted by atrial tissue in response to experimental or genetic hypertension. Recent studies have shown that ANF is produced and secreted by left ventricular tissue as well, and that ANF has direct effects on ventricular cardiac myocyte contraction. Since heart contractile function is altered during aging, and the senescent heart exhibits many other phenotypic and genotypic characteristics of the hypertensive heart, we tested the hypothesis that the atrial natriuretic factor (ANF) gene would be upregulated with advancing age. In addition we studied differences in the response of ANF mRNA levels to aortic constriction in hearts of young adult and senescent rats. Total RNA was isolated from left ventricles (LV) of male Wistar and Fischer rats aged 1.5-27 mo of age. Northern blots were probed with a radiolabeled cDNA probe synthesized by PCR using oligonucleotides complementary to the published sequence. The levels of mRNA coding for ANF increased progressively with advancing age in ventricles of both strains of rats. ANF mRNA abundance was 7-fold greater in ventricles of senescent compared to young adult rats. In freshly isolated ventricular myocytes, a similar pattern was observed. The induction of the ANF gene in ventricles of aortic-constricted rats was blunted with advancing age. Since ANF peptide is known to affect cardiomyocyte function, the age-associated increase in ANF gene expression may be of functional importance in the senescent heart.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00804-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cardiac Gene Expression During the Transition from Hypertrophy to Heart Failure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	M. Boluyt	NRC Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	L. O'Neill	Biologist	LCS, NIA
	E. Mayoux	Visiting Fellow (DOD 8/29/92)	LCS, NIA

COOPERATING UNITS (if any)

Boston VAMC, Boston, MA (O.H.L. Bing)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism(s) which contribute to the transition from compensated cardiac hypertrophy to heart failure have remained obscure. Our purpose was to determine whether some of the changes observed during the transition to heart failure might be regulated at the level of gene expression. The spontaneously hypertensive rat (SHR) served as a model for these studies. This model has recently been characterized in terms of functional and morphological features. At 18 mo of age some SHR rats develop clinical signs and symptoms of failure. By combining daily inspections with post-mortem morphological and pathological data, the SHR rats can be divided into those exhibiting failure and those in whom cardiac function is well compensated. Since the failing heart is characterized by impaired function and increased fibrosis, we studied levels of contractile protein and connective tissue mRNA. Total RNA was isolated from hearts of 18-24 mo normotensive Wistar-Kyoto (WKY), spontaneously hypertensive (SHR) rats with (F) and without (NF) evidence of heart failure. Relative mRNA levels for atrial natriuretic factor (ANF), fibronectin (Fn), α-skeletal (Sk) and α-cardiac (Ca) actin, and α- and ß-myosin heavy chain (MHC) were assessed. The transition from compensated hypertrophy to failure was accompanied by decreased contractile protein gene expression (α-MHC; 28% of SHR-NF value) and increased expression of fibronectin (301% of SHR-NF value). Expression of ANF was maintained at an elevated level compared to that of age-matched WKY (365%). The results suggest that the impaired function and increased fibrosis observed in failing hearts may be regulated, in part, at the level of gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00805-01 LCS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormal Myocyte Ca^{2+} Handling in A Rodent Model of Chronic Heart Failure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	E. Mayoux	Visiting Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	H.A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Compensated myocardial hypertrophy occurring in response to chronic pressure overload usually progress to a decompensated state, with myocardial depression and cardiac pump dysfunction. The mechanisms involved in the transition from the chronically adapted to the chronically failing heart remain unclear. A major reason has been a relative lack of animal models of stable hypertrophy. We have developed a practical and reproducible model of cardiac hypertrophy that progresses to chronic heart failure, within three months, by banding the ascending aorta of young rats. Gradual left ventricular overload occurs as the animals mature, leading to a compensated left ventricular hypertrophy and subsequent cardiac failure. Animals with cardiac failure, identified by clinical symptoms (increased respiratory rate), presented pathophysiological alterations consistent with heart failure: high lung weight, hypertrophy of the left atria, right ventricle, and right atria. In addition most of the failure animals were observed to have pleural effusions. The first physiological study on animals provided by our experimental model show that the contractile behavior of myocytes isolated from failing hearts tended to be different under stressful conditions. Myocytes from failing hearts had reduced rates of twitch shortening and rates of relenghthening, especially at high drive rates (2.5 Hz) or at elevated external calcium concentrations (6 mM), compared to control. Application of the different technology available in the laboratory, such as measures of intracellular calcium handling with Indo-1, molecular biology ..., to this model of progressive heart failure should be able to elucidate mechanisms that underlie the transition from compensated hypertrophy to chronic heart failure.

Laboratory of Cellular and Molecular Biology

This laboratory conducts fundamental research on some of the basic systems of molecular biology as well as studies designed to understand the biology of aging. The laboratory was not designed to operate in a pyramidal mode, in which the laboratory chief formulates a grand design. Rather it was organized to bring together sections led by investigators with a diversity of goals but also a community of interests. Every section in the laboratory has engaged in successful collaborations with other sections.

The Inorganic Biochemistry Section has conducted many studies on nucleic acid structure and function as well as a variety of studies on molecular structural changes in aging. A particular emphasis at the moment is the project on the mechanism of RNA synthesis at the site of internucleotide bond formation, leading to a model of structure in the active site of RNA polymerase that is compatible with the functions of the enzyme. A comprehensive theory has been developed for the high fidelity of transcription.

A Unit on In Vivo NMR has been created. It focuses on the physiology of the isolated perfused heart and lung, and is continuing the study on the metabolic effects of exercise with the Baltimore Longitudinal Study on Aging.

The Molecular Dynamics Section shares the commitment to structural studies with the Inorganic Biochemistry Section, and is actively collaborating on the RNA synthesis study. Its importance is signified by its title - the emphasis on the dynamics, i.e., molecular motion that occurs during - and is required for - biological function. The section is presently involved in characterizing the dynamics of the interaction of hemoglobin with oxygen and other ligands, having identified distal perturbations and subunit interactions across the $\alpha_1\beta_1$ interface as important components in the process. It is also engaged in studying the consequences of the discovery of enhanced hemoglobin oxidation rate under hypoxic stress resulting in the formation of superoxide.

The Macromolecular Chemistry Section has carried out a variety of activities designed to understand the molecular basis of drug action and to lead to the design of better drugs. Recent work has been focused on the cyclodextrin studies, and has led to the development of techniques for binding adducts to specific sites on the cyclodextrin molecule.

The Molecular Physiology and Genetic Section is dedicated to the study of the regulation of physiological functions during aging. The studies on age changes in hormone and transmitter action involve adrenergic receptors and are therefore related to the work of the macromolecular chemistry section. The section is also studying age changes in central nervous system responsiveness, behavioral biology, gene expression and the

biology of human longevity. It is also involved in determining whether caloric modification increases lifespan in primates as well as in previously studies rodents.

Following are some of the highlights of the research in each section:

INORGANIC BIOCHEMISTRY SECTION

Mechanism of Fidelity in RNA Synthesis

We have previously reported a mechanism for the achievement of fidelity in RNA synthesis that depends on the E. Coli RNA polymerase enzyme to differentiate between "correct" and "incorrect" bases on incoming substrate nucleoside triphosphates (NTPs). In this mechanism the enzyme acts as a switch, with Mg as the active component, to move the α-P into position for bonding a "correct" NTP onto the OH-terminal of the nascent RNA chain, or to move the α-P of an "incorrect" NTP away from such a favorable bonding position. In the latter case the γ-P is moved into proximity of an NTPase that destroys the NTP. Evidence for the mechanism is threefold: increase in Zn-Mg distance for "incorrect" (non-complementary) substrate, constancy of Mg-phosphate distances in an NTP with different conformations for "correct" and "incorrect" bases, and finally the association of an NTPase with the RNA polymerase.

Molecular Modeling Studies

We have now obtained molecular models for the differences in the geometry of interaction of an NTP in the growing RNA chain site (i) and another NTP in the incoming NTP site (i+1), where the NTP base is complementary or noncomplementary. The molecular modeling was done with the aid of a molecular modeling workstation and a molecular mechanisms software package. Metal-metal and metal-substrate distances obtained by NMR and EPR methodology as well as other structural information was used as constraints. Structures were obtained by energy minimization. This process produces a number of virtually identical structures for both the complementary and noncomplementary systems. In fact, a comparison of the complementary and noncomplementary systems shows qualitatively similar relationships, with the only major difference in the position of the triphosphate, which our theory had predicted to move between the two systems. The α-P is moved 0.5 Å away from the i-site OH in the noncomplementary system, enough to prevent bond formation. The best structure obtained for the noncomplementary systems also moves the γ-P a considerable distance from its position in the complementary system. All of these findings support our fidelity of transcription model, and enhance our understanding of the precise structural effects that lead to fidelity.

The NTPase and Fidelity

We have previously separated an NTPase activity from the RNA polymerase. Other laboratories had obtained evidence that such an NTPase activity associated with RNA polymerase is involved in assuring RNA synthesis fidelity. We have identified our NTPase as a protein of approx. 70 kD, about the same mass as Sigma subunit but distinct from it. This size similarity may account for the failure of others to separate the NTPase.

We have carried out studies to determine how the NTPase aids in assuring fidelity. The presence of single- or double-stranded polydeoxynucleotide template stimulates several fold the cleavage of noncomplementary NTP. NTPase-free RNAP holoenzyme incorporates noncomplementary NTP into long-chain RNA product at a higher rate than the parent, NTPase-containing RNAP. These results are all in line with the participation of the NTPase in the scheme represented by our model.

Studies on Yeast RNA Polymerase II

Preliminary atomic absorption studies indicated on the order of one tightly bound plus one (or more) weakly bound zinc ions per RNAP II molecule. The eukaryotic RNA polymerases in general are more difficult to study than the bacterial because of the greater number of subunits (10-12 vs. 5), greater variety of enzyme types (3 vs. 1) and poorer yield per cell. To provide adequate and pure enough enzyme for detailed studies on metal content and for magnetic spectroscopy, we are implementing preparation scale-up from the more usual few milligrams to several hundreds of milligrams. We are making good progress, simplifying certain steps and using HPLC methods.

IN VIVO NMR UNIT

NMR Studies of Aging in Cells, Organs and Animals

NMR spectroscopy is currently being used to study the phosphorus metabolism of peripheral muscle in BLSA subjects and other volunteers. Age-related and exercise-related effects are under investigation. Methodologic studies to further develop magnetization transfer techniques for kinetic measurements are also under active investigation.

Saturation transfer studies have been undertaken in an attempt to significantly improve the accuracy of kinetic measurements. We have found that experiments with spillover and incomplete saturation can be modeled by a modification of the Bloch equations used to describe transient saturation transfer. We have demonstrated that these experimental effects, when present to a degree commonly seen in in-vivo experiments, can lead to substantial errors in derived rection rates and spin-lattice

relaxation rates. We can therefore make improved measurements by using a more appropriate mathematical model.

Molecular Studies by Solid-State NMR

We have successfully elucidated detailed structural features of a nine-amino acid peptide found in the amyloid protein of Alzheimer's disease. These features may play a critical role in the stability of the amyloid protein, and therefore in the pathology of Alzheimer's disease. More basic work has included development of what is essentially a CPMAS version of TOCSY. We call this experiment DICSY, for dipolar correlation spectroscopy. Other ongoing research includes the theory and demonstration of CPMAS experiments i) to create rotational resonances at scaled chemical shift offsets ii) to explore dipolar interactions involving three nuclei and iii) to selectively invert one sideband manifold overlapping that of another nucleus.

Studies on the Amyloid Fragment Associated with Alzheimer's Disease

We have described the backbone structure of the nine-amino acid amyloid fragment discussed above to a resolution of approximately 0.2A. Our experiments reveal that a cis peptide bond is present between residues corresponding to gly-37 and gly-38 in β amyloid. This conformation may have an important role in the stability of amyloid plaques.

MACROMOLECULAR CHEMISTRY SECTION

Many medicines, and especially those recently developed by medicinal chemists and molecular biologists, cannot be effectively administered to humans: they are too water-insoluble, too prone to hydrolysis or tend to precipitate from parenteral formulations. Hydroxypropyl cyclodextrins are potent and non-toxic solubilizers which may help with some of these difficult problems. During this year the work in the Section addressed the characterization of hydroxypropyl cyclodextrins as mixtures of chemical individua and defined the parameters decisive in their preparation.

MOLECULAR DYNAMICS SECTION

Oxidation of Hemoglobin

Our recent studies on oxidation of hemoglobin has focused on a free radical intermediate which has been attributed to a superoxide partially displaced from the heme, but still retained in teh heme pucket. By detailed oxidation and electron paramagnetic resonance studies, it has been shown that oxidation

is correlated with a rearrangement in the ligand pocket which favors the displacement of an exogenous ligand by the distal histidine. It is this nucleophilic displacement which is responsible for the free radical intermediate.

Hemoglobin Mediated Erythrocyte Damage

We have previously demonstrated that superoxide is released from the erythrocyte as a result of the binding of hemoglobin to band 3. A detailed analysis of the reduction of nitroblue tetrazolium as a function of oxygen pressure indicates that the maximal formation of superoxide occurs at intermediate oxygen pressures with hemoglobin partially oxygenated. At lower oxygen pressures a perturbation of the conformation of hemoglobin bound to the membrane occurs. This process is further shown to increase the susceptibility for hemoglobin oxidation provided a suitable electron acceptor is available. These interactions produce crosslinking and proteolysis of the erythrocyte membrane proteins. This mechanism for producing hypoxic damage to erythrocytes and possibly other cells is in addition to the oxyradical mechanisms reported previously.

Erythrocyte Deformability

Oxygen delivery to the tissues requires that the erythrocyte deform in order to pass through the narrow pores in the capillary bed. A decrease in cell deformability, however, not only inhibits oxygen delivery, but because of a longer transit time through the capillary bed, results in a more hypoxic environment. With this in mind we have initiated studies to understand the factors which control erythrocyte deformability and to investigate possible changes with age. Preliminary studies indicate a decrease in cell deformability for older subjects. We have also found that the decreased deformability is exacerbated by erythrocyte swelling which is produced by oxyradical damage under hypoxic conditions.

MOLECULAR PHSIOLOGY & GENETICS SECTION

Drug Development for Cognitive Enhancement

Collaborating with Dr. Grieg, we have continued to analyze effects of novel cholinomimetic on learning performance. We tested the effects of a potent anticholinesterase, phenylphysostigmine, for reversing learning impairment induced by scopolamine, a muscarinic antagonist, in rats in the 14-unit T-maze and in mice in a Morris swim maze. This drug had a long duration of action (>1 hr) and was observed to reverse the learning impairment with a wide range of therapeutic efficacy with minimal observable side effects except at high doses (>10 mg/kg).

Nitric Oxide Involvement in Learning

Collaborating with Dr. E. London, we have continued to examine the involvement of the glutamatergic system in memory systems in general and in age-associated memory impairment in particular. To examine the intracellular signal transduction events in which the NMDA glutamate receptor is involved, we provided compounds to young rats which blocked the activity of nitric oxide synthase (NOS) and thus reduced production of nitric oxide (NO) which is thought to serve as a retrograde messenger enhancing neurotransmitter release. Young F-344 rats treated with N-methyl-L-arginine or N-methyl-L-arginine methyl ester (3.0-6.0 mp/kg) were impaired in learning the 14-unit T-maze.

Laminin and Laminin Receptors in Brain

Collaborating with Drs. M. Jucker, H. Kleinman, L. Walker,and D. Price, we have continued to examine the immunocytochemical profile of antibodies to a 110 kD laminin binding protein (LBP). Further work has demonstrated its utility for staining reactive astrocytes following brain injury, such as ischemia. Of major importance this year, however, was the identification of unique pathology in the brains of old C57BL/6J mice. This was identified as astrocytic inclusions primarily located in the hippocampus. These inclusions were positive to antibodies to LBP and to beta-amyloid as well as several other probes. Their exact structure and origin have yet to be identified but they might provide a potential model of a precursor condition for development of amyloid plaques.

Calorific Modification in Primates

After 5 years of 30% reduced dietary intake, juvenile and adult male rhesus monkeys of our initial group exhibit marked reduction in body weight compared to ad libitum fed counterparts. These animals appear to be somewhat different than those in our second group (now 4 years in the study) which do not exhibit such reductions in body weight despite reduced food intake. The second group also appears to be different on other biological parameters, most notably lower cholesterol levels and reduced levels of HDL2 cholesterol in dietarily restricted animals. Molecular genetic analysis of apolipoprotein subtypes in the first and second groups is currently underway in attempts to gain some insight into the metabolic basis for efficiency, or lack thereof, of dietary restriction on biological functions.

We have confirmed our preliminary observations that puberty (as indexed by increased circulating levels of testosterone) and skeletal maturation (as indexed by decreased levels of serum alkaline phosphatase) are delayed by 12-18 months in restricted animals. In addition, the progressive age related decrease in plasma DHEA levels may also be delayed or slowed by dietary restriction.

A number of cross sectional age differences continue to be observed. Recently, age associated increases in pentosidine adducts in skin collagen have been detected. These appear to be approximately twice as great in squirrel monkeys as rhesus; roughly proportional to maximal lifespan. Skin biopsies have also been obtained in order to culture fibroblasts for analysis of growth factor responsiveness, telomere length shortening, clonal analysis, glutathione levels, and calcium mobilization (the last two are also being determined in lymphocytes). With the completion of our cohort of 60 female rhesus monkeys we have also observed a cross sectional age-related decrease in mitogen stimulations of T-lymphocyte DNA synthesis roughly analogous to that already seen in males.

Calcium Dependent Signal Transduction and Aging

We are currently testing the hypothesis that impaired coupling/uncoupling of receptors and G-proteins during aging is due to alterations in the membrane environment. A number of membrane active agents including alcohols, H_2O_2, and detergents cause impaired alpha-adrenergic and muscarinic cholinergic signal transduction in rat parotid cell aggregates which appear to be analogous to that seen during aging. Unlike aging which exerts preferential effects on alpha-adrenergic responsiveness in these cells, however, the above treatments seem to cause roughly equal impairment in alpha-adrenergic and cholinergic stimulation of IP_3 production and calcium mobilization. Since our previous work suggested that different G-proteins and/or phospholipases C might be utilized for alpha-adrenergic and cholinergic signal transduction, age changes in the membrane environment would appear to be quite specific.

Efforts continue to isolate "ternary complexes" of agonists receptors and G-proteins. We have succeeded in solubilizing muscarinic receptor complexes which are GTP sensitive for agonists but not antagonists, suggesting that these may be ternary. Unfortunately, however, the time required to separate them on sucrose gradients (about 2 hours) appears to allow more than 80% dissociation of the ^{3}H-labeled agonists.

Steriod Action during Aging

We continue to test the hypothesis that loss of steroid receptors and altered genomic association result in functional impairments during aging. Preliminary experiments indicate that basal secreted levels of apolipoprotein A1 mRNA are elevated in hepatocytes from aged rats concomitant with increased basal secretion of the protein. We are currently attempting to determine whether altered estrogenic regulation of the apolipoprotein A1 gene results in impaired stimulation of secretion in aged cells.

The Striatum, Hippocampus and Second Messenger Function in Senescence

Earlier findings indicated that deficits in muscarinic enhancement in K^+-evoked DA release (K^+-ERDA) in the striatal slice appear to be the result of specific alterations early in muscarinic ST. Current findings suggest that these declines are the result of specific, structural (possibly free radical induced) changes in the membranes which compromise muscarinic receptor (mAChR)-G protein interactions. This research has shown that the incubation of striatal slices from senescent animals with the fluidizing and methylating agent, adenocyl-methionine, (SAM) will restore oxotremorine-enhanced K^+-ERDA, while incubation of striatal slices from young animals with cholesterol will significantly decrease oxotremorine-enhanced K^+-ERDA, and that membrane viscosity increases as a function of age.

As further evidence of mAChR-G protein deficits in aging, We have observed age-related deficits in carbachol-stimulated low K_m GTPase in striatum and hippocampus. Similar deficits have been observed in basal ganglia from Alzheimer's victims. However some restoration of this activity can be achieved in rat striata by increasing membrane fluidity with SAM.

Loss of Striatal D_2 Receptor mRNA in Senscence

Previous experiments revealed an age-related 50% loss of D_2 mRNA that was partially the result of cell loss and decreased synthesis. Present research indicated that the decreased synthesis was the result of specific decreases in the synthetic competence of old nuclei for the production of D_2 mRNA. Other mRNA species (eg., actin) did not show age-associated declines.

Complementary work continued in our tissue culture model, which has revealed selective toxicity of striatal dopamine D_2-receptor containing neurons to the excitatory amino acid analog kainic acid and DA itself. The loss of cells *in vitro* paralleled the decreases seen *in vivo*, which indicated the potential for further investigation into the mechanisms of the age-related loss of these cells using this tissue culture system.

Stability of Proper Gene Expression

Our collaboration with Dr. Arifa Khan of the National Inst. of Allergy and Infectious Diseases, NIA, has continued, in which we have been studying the possible age-dependent expression of endogenous MuLV-related sequences in mouse and endogenous retroviral sequences in human tissues. These retroviral studies indicate that endogenous viral gene expression is not static but rather is highly variable throughout the entire life span of an animal.

Evaluation of the spin-trap agent, N-tert-butyl-α-phenylnitrone (PBN).

Collaborative efforts within our section (Dr. D. Ingram, G. Roth and J. Joseph) have been on evaluating the potential pharmacological effects of the spin-trap, PBN, to reverse physiological aging. Our laboratory has focused on determining if PBN reverses the age-dependent decrease in multicatalytic protease activity. Preliminary results indicate that the protease activity does decrease with age and that PBN is effective in increasing proteolytic activity in old animals (rats) to that approaching young animals. Recent evidence indicates also that activity of the protease is dependent on source and age of substrate preparation. We also have evidence indicating that PBN given orally to old mice may increase life span. Other studies have indicated the possibility that PBN may act by a nitric oxide delivery agent to specific neuronal sites. We now have evidence that PBN does degrade in the presence of ultraviolet light or hydroxyradicals to form nitric oxide compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00044-19 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metal Ions and Information Transfer; mechanism of RNA Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gunther L. Eichhorn, Chief, LCMB,NIA

Others: James J. Butzow, Commissioned Officer, IBS LCMB NIA
Peter P. Chuknyisky, Sr. Staff Fellow, IBS LCMB NIA
Patricia Clark, Research Chemist, IBS LCMB NIA
Carl Garland, Visiting Fellow, IBS LCMB NIA, DOD 10/91
Chris Janzen, ITRA Fellow, IBS, NIA, EOD 9/91

COOPERATING UNITS *(if any)*

Israel Institute for Biological Research (D. Waysbort); Univ. of Western Ontario (S.J. Karlik); MDS (LCMB) (J. Rifkind, A. Levy), Centre for Cellular and Molecular Biology; (D. Chatterji); Univ. of Utah (L. Powers)

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of metal ions on nucleic acids structure and function have been determined. The primary focus now is on the mechanism of RNA synthesis at the active site of RNA polymerase. The geometry of interaction at the active site of the enzyme is being probed during the transcription process to determine how this geometry changes during transcription and affects its mechanism. Evidence has been obtained that the enzyme assures fidelity in copying the genetic code by conformation changes sensitive to complementarity or non-complementarity between DNA base and the base on the incoming nucleoside triphosphate. A comprehensive mechanism of fidelity has been developed that allows a Mg(II) switch to effect or prevent incorporation of the right and wrong substrate, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 0381-02 LCMB

PERIOD COVERED

October 1, 1991 to Septmeber 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR Studies of Aging in Cells, Organs, and Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard G. S. Spencer, Unit Chief, In-vivo NMR
Others: Gunther L. Eichhorn, Chief, LCMB
Jerome L. Fleg

COOPERATING UNITS *(if any)*

Cardiovascular Section, Clinical Physiology Branch, NIA

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NMR spectroscopy is currently being used at the NIA to study the phosphorus metabolism of peripheral muscle in BLSA subjects and other volunteers. Age-related and exercise-related effects are under investigation. Methodologic studies to further develop magnetization transfer techniques for kinetic measurements are also under active investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00382-02 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Studies by Solid-State NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard G. S. Spencer, Unit Chief, In-vivo NMR

Others: Professor Robert Griffin (MIT)

Professor Peter Lansbury (MIT)

COOPERATING UNITS *(if any)*

MIT Department of Chemistry, and Francis Bitter National Magnet Laboratory

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have successfully elucidated detailed structural features of a nine-amino acid peptide found in the amyloid protein of Alzheimer's disease. These features may play an critical role in the stability of the amyloid protein, and therefore in the pathology of Alzheimer's disease. More basic work has included development of what is essentially a CPMAS version of TOCSY. We call this experiment DICSY, for dipolar correlation spectroscopy. Other ongoing research includes the theory and demonstration of CPMAS experiments i) to create rotational resonances at scaled chemical shift offsets ii) to explore dipolar interactions involving three nuclei and iii) to selectively invert one sideband manifold overlapping that of another nucleus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00047 22

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Relationships in Hemoglobin and Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Joseph M. Rifkind Chief, MDS MDS LCMB NIA

Others: Abraham Levy, Sr. Staff Fellow, MDS/LCMB/NIA; Omoefe Abugo, Visiting Fellow, MDS/LCMB/NIA; J. Kinsella, NIA/LCS/CFS; P.T. Manoharan, Indian Inst. of Tech., Madras, India; V.S. Sharma, U. CA-San Diego, La Jolla, CA; V. MacDonald, Letterman Army Inst. of Res., San Francisco, CA; D. Danon, Waldenberg Center for Gerontological Studies; D. Ingram, NIA/LCMB/MPGS; R. Rao, U. of Maryland, Baltimore, MD;

COOPERATING UNITS *(if any)*

NIA/LCS/CFS; Indian Inst. of Tech., Madras, India; U. CA-San Diego, La Jolla, CA; Letterman Army Inst. of Res., San Francisco, CA; Waldenberg Center for Gerontological Studies; NIA/LCMB/MPGS; U. of Maryland, Baltimore, MD

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Dynamics Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the mechanism involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. Emphasis is placed on ways in which these functions are impaired and change with age. These studies have focused on the oxidation of hemoglobin, which produces nonfunctional hemoglobin and the simultaneous release of oxyradicals. The enhancement of these oxidative processes under hypoxic conditions is being explored as a possible source of tissue and organ damage, which would be exacerbated during aging. Studies are also included which are directed at the stability of the entire erythrocyte and the erythrocyte membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00046-22 LCMB

PERIOD COVERED

October 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Recognition of Lipids and Lipophiles by Cyclodextrin Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Josef Pitha, Chief, Macromolecular Chemistry Section, NIA/LCMB

Others: Bengt Lindberg, Ph.D., University of Stockholm
Makoto Komiyama, Ph.D., University of Tokyo
Kazuaki Harata, Ph.D., Research Institute of Polymers
Kaneto Uekama, Ph.D., Tsukubal and Kumanoto University

COOPERATING UNITS *(if any)*

University of Stockholm; University of Tokyo; Research Institute of Polymers; Tsukubal and Kumanoto University

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Macromolecular Chemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hydroxypropyl Cyclodextrins, chemical derivatives of glucose oligosaccharides, which were originated in the section, form water soluble complexes with lipophiles. Since these derivatives are also non-toxic they have been used as drug solubilizers with enough of success to qualify them for consideration to be included in the next edition of US Pharmacopea/National Formulary. Since hydroxypropyl cyclodextrins are amorphous mixtures of chemical individua and thus difficult to characterize, the present work of the section has been focused on the effects which preparative conditions may have on their properties. The reactivity of cyclodextrins was investigated, the mixtures subjected to a detailed analysis, several components of these and of similar mixtures were isolated as chemical individua, crystallized and their crystal structure determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00302-9 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Physiological Functions During Aging: III. Behavioral Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Donald K. Ingram, Ph.D., Research Psychologist, MPGS, LCMB, NIA

Others: E. Bresnahan, R. Cutler, R. Fanelli, P. Garofalo, N. Grieg, S. Iijima, J. Joseph, M. Jucker, H. Kametani, J. Knapka, S. Kobayashi, E. London, M. Ordy, M. Pontecorvo, D. Price, G. Roth, E. Spangler, L. Walker, L. Williams.

COOPERATING UNITS *(if any)*

Essex Community College; Tokyo Metropolitan Institute of Gerontology; Nat'l Inst. Dental Res; Fisons Corp; Upjohn Company, JHU Medical School; Nat. Ctr. Res. Resources; Nova Pharmaceuticals; NIDA; Miles Pharmaceuticals; Tokyo University

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.3	4.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to assess the effects of aging at a behavioral level of analysis, to identify neurobiological mechanisms associated with these effects, and to test interventions which might alter age-related performance decrements. Rodent models are tested in a battery of sensorimotor and learning/memory tasks. Neurochemical and neurohistological assays are conducted to determine neurobiological correlates of functional losses. Interventions include dietary restriction, exercise, various pharmacologic treatments and neurotrophic factors. Multiple genotypes are examined to determine possible genetic involvement in the pattern of age-related behavioral impairment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00304-6 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Physiological Functions During Aging: V. Assessment of Primate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB,NIA
D.K. Ingram, Ph.D., Research Psychologist, MPGS, LCMB, NIA
R.G. Cutler, Ph.D., Research Chemist, MPGS, LCMB, NIA
M.A. Lane, Ph.D., IRTA Fellow, MPGS, LCMB, NIA

Others: J. Knapka, D. Barnard, R. Andres, R. Weindruch, W. Ershler, D. Danon, B. Flynn, L. Olsen, N. Wolf, P. Rabinovitch, C. Harley, M. Reynolds, V. Monnier, S. Ball

COOPERATING UNITS *(if any)*

Department of Medicine, University of Wisconsin, Madision, WI, Department of Pathology, University of Washington.

LAB/BRANCH

Laboratory of Celluar and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Resarch Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is attempting to determine whether caloric modification of the diets of Rhesus and squirrel monkeys can affect aging rate as assessed by various physiological, biochemical and behavioral indices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00301-9 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Physiological Functions During Aging: I. Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB, NIA

Others: B. Baum, R. Villalobos-Molina, A. Richardson

COOPERATING UNITS *(if any)*

Patient Care Branch National Institute of Dental Research; V.A. G.R.E.C.C., University of Texas, San Antonio

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	1.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is mainly involved in elucidating those mechanisms by which the ability of hormones and neurotransmitters to regulate physiological functions is altered during aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00306-4 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reg. of Physiological Functions During Aging:II. Neurotransmitter Responsiveness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: J.A. Joseph, Research Pharmacologist, MPGS, LCMB,NIA
G.S. Roth, Chief, MPGS, LCMB, NIA

Others: M. Anson, R. Culter, Ph.D., G. Mesco, Ph.D., K. Yamagami, Ph.D., D.K. Ingram, Ph.D., P. Mason, Ph.D.

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.999	0.0001

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project attempts to understand those mechanisms involved in age related changes in central nervous system responsiveness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00303-8 LCMB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Physiological Functions During Aging: IV. Genes and Longevity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. G. Cutler, Ph.D., Research Chemist, LCMB, NIA

Others: D.K. Ingram, G.S. Roth, A. Ayala, A.S. Khan, G. Cao, K. Kitani, H. Alessio, I. Zs.-Nagy.

COOPERATING UNITS *(if any)*

Miami Univ., Ohio: LMM, NIAID, Bethesda, Tokyo Metrop. Inst. Gerontol.; Verzar Inst. Exp. Geront.

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our research has focused on assessing the possible role oxidative stress may have as an initiator of aging and on mechanisms reducing oxidative stress as possible determinants of longevity. Methods to detect and measure oxidative damage are essential to these studies. We have developed a new assay to measure total oxygen radical absorption capacity (ORAC) of serum. Using this assay, we have found that the ORAC value of serum increases in direct proportion to life span of different mammalian species but appears not to change with age. The spin-trap agent, N-tert-butyl-α-phenylnitrone (PBN) has been reported to reverse a number of age-related effects, including a decrease in oxidative stress state. Preliminary results in our laboratory indicate that PBN may stimulate molecular renewal rate (multicatalytic protease activity) in old mice and rats and possibly extend life span of old mice. The mechanisms of PBN action may involve delivery of nitric oxide to specific neurological sites in the brain.

Diabetes Unit, Laboratory of Clinical Physiology

Type II diabetes constitutes greater than 90% of patients with diabetes in the United States and essentially all diabetes in the elderly. The incidence of diabetes increases progressively with age and with obesity. Currently available therapeutic measures (changes in lifestyles which are mainly dietary and exercise; sulfonylurea drugs; insulin) have major shortcomings so that most patients with diabetes are not well controlled. It is widely believed that the complications of diabetes such as kidney failure, blindness, disability, and premature death are the results of prolonged periods of hyperglycemia. The factors responsible for type II diabetes are abnormal insulin secretion from islets of Langerhans in the pancreas and impaired sensitivity to insulin peripheral tissues (e.g. liver, muscle). The major goal of the Diabetes Unit is to devise new approaches to the treatment of type II diabetes that are safe and effective, especially in elderly patients with diabetes.

The specific objectives of our studies were two fold. First, define regulatory signals controlling pancreatic insulin secretion and, second, increase our understanding of insulin resistance at the molecular level.

Several complementary approaches have been devised with the long-term aim of improving the early insulin secretion by giving agents that synergize with glucose in stimulating insulin release.

1.) We have conducted a systematic evaluation of the effects of two insulinotropic agents added alone or in combination with glucose and amino acids, using a long-term primary culture of islets of Langerhans from rodent pancreas. These naturally-occurring peptides, gastric inhibitory peptides (GIP) and glucagon-like peptide (GLP), are each released from a distinct population of cells of the gastrointestinal tract after a mixed meal and have been shown to promote insulin secretion by a mechanism that requires elevated blood glucose.

2.) Complementary studies of the interaction of these two peptides will be carried out in the General Clinical Research Center with a wide age range of human subjects. We already have IRB approval for this protocol. The hyperglycemic glucose clamp technique will be used to assess the effects of these peptides on insulin secretion and action at a constant level of blood glucose. Likewise, a similar protocol is also under way with rhesus monkeys (normal and diabetic, thin and obese, old and young) in collaboration with Dr. Barbara Hansen and her colleagues at University of Maryland.

3.) Recent data have suggested that GLP receptors are only expressed in the islets of Langerhans'. Therefore, we propose to characterize the GLP receptor at the level of binding parameters and investigate potential interactions with G proteins, identify activated signal transduction pathways, and study the structure-function requirements for ligand binding and activation of regulatory cascades. Based on these results, the cloning of the GLP receptor will be initiated in order to define the molecular requirements for regulation of receptor function and expression.

4.) Reg gene (regenerating gene) recently characterized from regenerating adult rat pancreatic islets may be important in determining how the B-cells respond to acute and chronic injury in diabetes as well as to the aging process. We predict that the Reg gene expression will be altered during the period of B-cell destruction (i.e. Type I diabetes) and B-cell dysfunction (i.e. aging or type II diabetes). We used molecular techniques to measure mRNA levels of Reg in normal pancreas of rodents at different ages. The results of our initial studies indicated an age-dependent alteration in the expression of the REG gene that paralleled the level of insulin mRNA. In contrast, ß-actin and elastase I mRNA exhibited an increased expression with age.

Another major research effort for the Diabetes Unit consisted of the identification of protein tyrosine phosphatases that dephosphorylate the activated form of the insulin receptor. This dephosphorylation reaction terminates the early steps in insulin action. By intervening at the level of these phosphatases one could prolong the state of activation of the insulin receptor, thereby alleviating many of the symptoms of diabetes. We have synthesized novel reactants and tested them for their ability to modulate the dynamics of insulin receptor phosphorylation/dephosphorylation. Some of these peptides appeared to specifically regulate insulin receptor kinase and tyrosine phosphatase activities.

All these studies provide new directions for the research and development of safe and effective therapeutic interventions for the treatment of older patients with diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00213-02 LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tyrosine phosphatases and insulin resistance in the aged

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Bernier Senior Staff Fellow LCP, NIA

Other:

A.S. Liotta Expert LCP, NIA

COOPERATING UNITS *(if any)*

Dr. Jesse Roth, Director of the Division of Geriatric Medicine & Gerontology, Johns Hopkins University School of Medicine

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at identifying important phosphatases that dephosphorylate the activated form of the insulin receptor, thereby terminating the early steps in insulin action. By intervening at the level of these phosphatases one could approach the problem of insulin resistance in maturity onset diabetic states.

These studies have required the preparation of several new reagents and optimization of cellular systems. Modern techniques in peptide/protein chemistry, receptor biochemistry and mammalian cell culture were used successfully to study the dynamics of insulin receptor phosphorylation/dephosphorylation. Several novel peptides were synthesized and tested for their ability to modulate insulin receptor activity. Among them two sets of tyrosine-modified peptides appeared to specifically regulate insulin receptor kinase and tyrosine phosphatase activities.

These studies provide new directions for the research in both the identification and inhibition of insulin receptor phosphatase, as well as the study of other aspects of the insulin signal transduction pathway. Our long term goal is the development of safe and effective therapeutic interventions for the treatment of older patients with diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00214-02 LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of an in vivo model system to investigate NIDDM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Egan Senior Staff Fellow LCP, NIA

Other:

J. Roth Special Volunteer LCP, NIA

COOPERATING UNITS *(if any)*

A.R. Shuldiner, Johns Hopkins University School of Medicine, Division of Geriatric Medicine and Gerontology

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been investigating the use of an in vivo model system, to study potential agents for use in non-insulin requiring diabetes mellitus (NIDDM). Dr. B. Hansen, University of Maryland, has an extraordinary well characterized rhesus monkey model of this disease. We therefore gave two peptides, GIP and GLP, both known to have insulinotropic effects in other systems, to these monkeys. We ascertained the dose required to give half maximum insulinotropic effects. We showed that even the NIDDM monkeys can get an insulinotropic effect if given enough of these agents. We showed the glucose threshold required for insulin release by GLP. Further studies are planned using combinations of these peptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00876-01 LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regenerating (REG) gene: A paracrine B-cell growth factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Perfetti	Visiting Associate	LCP, NIA
Other:			
	J. Egan	Senior Staff Fellow	LCP, NIA
	A.R. Shuldiner	IPA	LCP, NIA

COOPERATING UNITS *(if any)*

Dr. Jesse Roth, Director of the Division of Geriatric Medicine & Gerontology, Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recently, Terazono et al. isolated and characterized a cDNA from a regenerating rat pancreatic islet-derived library, designated REG (for regenerating gene) that was over expressed in regenerating islets and underexpressed when endogenous insulin secretion was suppressed. Others have identified Reg (also known as pancreatic stone polypeptide) in exocrine pancreas, suggesting that Reg may be a paracrine growth factor for B-cells and may play a role in the ß-cells response to injury.

Type I diabetes is characterized by autoimmune destruction of pancreatic ß-cells, while type II diabetes is characterized by abnormalities in insulin secretion and insulin action, or with the possible augmented loss of insulin secretory capacity with aging. We hypothesize that the REG gene may be important in ß-cell response to autoimmunity (Type I diabetes), ß-cell dysfunction (Type II diabetes) and in aging. It is hoped that these studies will further our understanding of how ß-cells respond to injury. In addition to possible providing a new marker for progressive ß-cell dysfunction and injury in diabetes, these studies may pave the way for new therapeutic strategies that alter REG gene expression in pancreas for the prevention or treatment of diabetes.

We plan to use molecular techniques to measure mRNA levels of REG in the pancreas of rodents during the normal aging process, and in rodent strains with genetic forms of type I diabetes (NOD mouse, BB rat), and type II diabetes (db/db mouse, fa/fa rat).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00877-01 LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular mechanisms of insulin secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Montrose-Rafizadeh Staff Fellow LCP, NIA

Others:

J. Egan Senior Staff Fellow LCP, NIA

J. Roth Special Volunteer LCP, NIA

COOPERATING UNITS *(if any)*

A.R. Shuldiner, Johns Hopkins University School of Medicine, Division of Geriatric Medicine and Gerontology

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Type II diabetes is a health problem of 20% of the elderly population in the U.S. The aims of this project are: to define regulatory signals controlling insulin secretion from beta cells of the pancreas; to study the mechanism of action of gastrointestinal hormones called incretins (e.g. glucagon-like peptides [GLP] and glucose insulinotropic peptide [GIP]) on stimulation of insulin secretion; and to study directly changes in insulin secretion during aging.

These studies have required the development of an in vitro system for the study of insulin secretion. Freshly isolated pancreatic beta cells and several pancreatic insulinoma cell lines were screened using reverse hemolytic plaque assay and radioimmunoassay for insulin. Freshly isolated beta cells were used to study the effect of extracellular matrix prepared from young and old animals on the insulin secretion. The best insulinoma cell line was chosen to study the effect of incretins such as GLP and GIP on insulin secretion. The information on incretin effects obtained from these in vitro study can now be applied to in vivo systems such as studies in monkey and in man.

The GLP receptor has been cloned from rat pancreas and the clone is available to us. Transfection of GLP receptor sequences into different cell lines will allow us to study further the signal transduction involved in GLP action. Further, the screening of peptides, with structural similarity to GLP will allow the understanding of structure-function relationship and the development of new therapeutic agents for type II diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00878-01 LCP

PERIOD COVERED

March 15, 1992 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Screening for mutations in target genes causing type II diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. S. Celi	Visiting Fellow	LCP, NIA
Other:			
	A. R. Shuldiner	IPA	LCP, NIA
	J. Roth	Special Volunteer	LCP, NIA

COOPERATING UNITS *(if any)*

Johns Hopkins University School of Medicine, Division of Geriatric Medicine & Gerontology

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutations in the insulin receptor gene are known to be a cause of some form of extreme insulin resistance. In a few patients similar mutations have been associated with the mild to moderate forms of insulin resistance observed in patients with NIDDM. It is currently unknown how wildly present this defect is. At present the PCR methods used to screen for point mutations (DGGE, SSCP, heteroduplex analysis) are extremely sensitive and allow researchers to study relatively large populations. However, these methods will fail to detect a partial or complete deletion . The methods currently used to screen for gene deletions (Southern blot analysis, RFLPs, quantitative Southern blot analysis) are troublesome, time consuming and require a large quantity of genomic DNA.

We plan to use a novel PCR method developed in our laboratory to screen for gene deletions in a large population of NIDDM patients from the greater Baltimore area as well as populations with a high prevalence of NIDDM (Pima native Americans, Nauruans, Mexican Americans).

ENDOCRINOLOGY SECTION, LCP, ANNUAL REPORT 1991
ABBREVIATED SECTION SUMMARY:

Clinical Studies- Aims: (1) delineating the effects of aging on regulation of hormone secretion; (2) elucidating whether hormonal alterations contribute to changes associated with "normal" aging; and (3) investigating the extent to which hormonal intervention can reverse or restore age-related metabolic and somatic alterations.

Female reproductive hormones & the menopause: Important questions with regard to menopausal hormone replacement therapy (HRT) include (a) what are optimal route, dose, agents, schedule and duration of treatment, and target populations to achieve the optimal risk/benefit ratio and (b) what are the mechanisms of action of HRT at bone and other tissues? In a study comparing the effects of estrogen replacement by oral vs. transdermal routes on GH & IGF-I and body composition & body fat distribution, we have found: (1) DEXA estimates of body composition comparable to those obtained by hydrodensitometry; (2) an age-independent decrease in GH secretion attributable to android habitus; (3) Oral estrogens, tend to increase and transdermal estrogens to decrease spontaneous GH levels; (4) Oral, but not transdermal estrogens significantly decrease basal and stimulated IGF-I; (5) transdermal estrogens had more effect to reduce IGF binding protein 3 than did oral estrogens. In another study of the effects of combination estrogen/progestin therapy on bone, lipids, and clinical symptoms in women >65, most women report minor breast tenderness as the only adverse effect. Four women receiving estrogen-progestin co-therapy experienced vaginal bleeding within the first three months.

Growth hormone and IGF-I: Many normal old people have reduced serum levels of IGF-I and GH secretion compared those in with young men and women. GH has been shown to stimulate nitrogen, calcium, and phosphate retention, increase muscle and bone mass and reduce percent body fat both in GH deficient young and elderly patients. We are investigating the relationship of GH deficiency to aging and testing the effectiveness of alternatives to recombinant human GH treatment. We have found reductions in pulsatile GH secretion and serum IGF-I levels in healthy older men, which functions increased into the range seen in normal young men after either injections or constant infusion of GHRH daily. In another study the amino acids arginine and lysine administered orally as a 50:50 mixture did not increase GH and IGF-I secretion in older men. Further studies are planned investigating the longer term clinical effects of raising GH and IGF-I and the effectiveness of alternative GH-elevating agents.

Cell Physiology , Biochemistry, and Molecular Studies- Aims: (1) to investigate mechanisms responsible for altered secretion of pituitary hormones with aging; (2) to investigate the mechanisms of interaction of GH, IGF-I, and sex steroid hormones in bone and muscle of young vs. old rodents.

It is known that gonadotropin (luteinizing hormone, LH; and follicle stimulating hormone, FSH) and GH secretion are profoundly reduced in old rats. Recent observations suggest that long vs. short term LH release may proceed through separate mechanisms which are differentially affected by aging. We are investigating alterations in the mechanisms of membrane transduction of regulatory signals in enriched gonadotropic cell populations prepared by centrifugal elutriation. We have shown (1) significant changes in the levels of ß-subunit (35K) which regulate the activity of α-subunits without changes in their levels, activate K^+ channels, and possibly have an effect (direct or indirect) on Ca^{++} channels; (2) an age- related decrease in levels of $G_{\alpha i3}$, the GTP regulatory subunit that putatively inhibits adenylyl cyclase, and (3) a significant increase in the levels of $G_{\alpha q}$ which is involved in the activation of phospholipase C, the enzyme generating IP_3 and diacylglycerol required for hormone release.

Placebo-controlled studies of the effects of 5 or 10 days of administration of GH, testosterone, or both in young and old intact male mice on gene activation in various tissues have been initiated. Initial results show increased total mRNA in tissues from GH treated animals. Molecular probing for mRNA's for growth factors, growth factor receptors and binding proteins, and tissue-specific differentiation factors and structural proteins is underway.

Z01 AG 00281-02 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones, Hormone Receptors, and Aging. IV. Hormone Replacement in Menopausal Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

S. Mitchell Harman, M.D.,Ph.D., Acting Section Chief, ES, LCP, NIA
Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA
Marc R. Blackman, M.D. Guest Scientist, ES, LCP, NIA
Janet Vittone, M.D., Guest Scientist, ES, LCP, NIA
Robin Roberson, B.S., Chemist, ES, LCP, NIA
Allison Campfield, Summer Student, ES, LCP, NIA

COOPERATING UNITS (if any)

Dr. Jordan Tobin, Chief Applied Physiology Section, LCP, NIA
Depts. of Medicine, Francis Scott Key Medical Center and Johns Hopkins University School of Medicine.
Dept of Obstetrics & Gynecology, Francis Scott Key Medical Center

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects X (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a group of healthy postmenopausal women whose body fat distribution and body fat mass independently range from lower (gynoid) to upper (android) distribution and from lower (lean) to higher (obese) percent fat we have found a trend to greater percent body fat with increasing age despite the absence of a significant relationship between age and body mass index. Data also suggest that dual energy x-ray absorptiometry yields estimates of body composition comparable to those obtained by hydrodensitometry. There were independent inverse correlations of waist to hip ratio ($p<0.03$), but not of age or body mass index, with 12 hour pulsatile GH levels and GHRH-stimulated GH peaks. These findings suggest an age-independent decrease in GH secretion attributable to android habitus in postmenopausal women. Oral estrogen treatment, produced a trend to increased spontaneous GH levels, which was significant only for the area under the peaks. Transdermal estrogens tended to decrease mean GH peak amplitude, and to increase 12 h mean GH. Oral, but not transdermal, estrogens significantly decreased basal and stimulated IGF-I, while transdermal estrogens had a greater effect on reducing IGF Binding Protein-3 than did oral estrogens. In another ongoing study of healthy women age 65-75 y treated with constant combined oral estrogen with and without low dose daily progestin, minor breast tenderness has been the only adverse effect reported to date. Four women receiving estrogen-progestin co-therapy experienced vaginal bleeding within the first three months of study requiring an increase in progestin dose.

Z01 AG 00013-17 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones, Hormone Receptors, and Aging. III. Aging and Human Endocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

S. Mitchell Harman, M.D.,Ph.D., Acting Section Chief, ES, LCP, NIA
Marc R. Blackman, M.D. Guest Scientist, ES, LCP, NIA
Emiliano Corpas y Cobisa, M.D., Ph.D., Visiting Scientist, ES, LCP, NIA
Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA
Marco Piñyero, B.S., M.S., Sr. Research Officer, ES, LCP, NIA
Robin Roberson, B.S., Chemist, ES, LCP, NIA

COOPERATING UNITS (if any)

Reubin Andres, M.D., Chief, Metabolism Section & Branch Chief, LCP, NIA
William Adler, M.D., Chief, Immunology Section, LCP, NIA
Depts. of Medicine, Francis Scott Key Medical Center and Johns Hopkins University School of Medicine.

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.6	0.5

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects O (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A study of 24 hr pulsatile GH secretion, acute GH response to iv GHRH, and plasma IGF-I measurements in older men, before and after two weeks of either twice daily sc GHRH injections or constant sc GHRH infusions by portable pump, each repeated at 2 different doses, has been completed. We demonstrated that pulsatile GH secretion and serum IGF-I levels were low in older men, and increased into the range seen in normal young men after treatment with 2 mg of GHRH by injection and either dose by infusion. Diurnal pulsatile secretory variation appeared more physiological with GHRH injections than during infusions, in that infusion tended to raise the interpeak (basal) GH levels and to diminish amplitude of nocturnal GH secretory peaks. GHRH treatment also resulted in apparent phosphate retention, suggesting an increase in protein synthesis. There were no adverse effects. A study of 2 weeks of orally administered 50:50 arginine/lysine mixture vs. placebo on GH and IGF-I secretion in older men has shown no effects on spontaneous GH secretory pattern, GH responses to GHRH, and serum IGF-I levels, suggesting that oral amino acids may not be a useful GH-stimulating intervention in the elderly.

ZO1 AG 00023-16 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones and Aging. Hypothalamic-Pituitary Function in Experimental Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

S. M. Harman, M.D., Ph.D., Section Chief, ES, LCP, NIA
M. R. Blackman, M.D. Guest Scientist, LCP, NIA
Marco Piñeyro, BS, M.S., Sr. Research Officer, LCP, NIA
Robin Roberson, B.S., Chemist, LCP, NIA
David Shock, B.S., Biologist, LCP, NIA
Zhong-Ding Lu Ph.D., Visiting Scientist, LCP, NIA
Michael R. Greisman, Summer Student, LCP, NIA

COOPERATING UNITS (if any)

Dept. of Medicine, Francis Scott Key Medical Center and Johns Hopkins University School of Medicine
George S. Roth, Ph.D., Section Chief, MPGS, LCMB, NIA
Atsushi Miyamoto, Ph.D. Visiting Scientist MPGS, LCMB, NIA

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.3	1.8

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In studies of pituitary cell membrane signal transduction, we have looked for age-related changes in the levels of GTP regulatory units using specific antibodies against $G_{\alpha S}$, $G_{\alpha i1\&2}$, $G_{\alpha i3}$, $G_{\alpha q}$, and ß-subunits. No significant changes due to age were found in the levels of $G_{\alpha S}$. Significant changes are seen in the levels of ß-subunit (35K) which predominates over the 36K (9:1). It is important to consider the levels of the ß-subunits since it is now clear that they regulate α-subunit activity without changes in α-subunit levels, activate K^+ channels, and possibly have an effect, direct or indirect on Ca^{++} channels. There was a significant age- related decrease in levels of $G_{\alpha i3}$, the GTP regulatory subunit that putatively inhibits adenylyl cyclase, and a significant increase in the levels of $G_{\alpha q}$ which is involved in the activation of phospholipase C, the enzyme generating IP_3 and diacylglycerol required for hormone release. In a new initiative on the effects of age on tissue responses to GH and testosterone in an animal model, we have cloned and successfully used labeled cDNA probes to quantify mRNA's for EF1-α in muscle and other tissues using Northern blotting and mRNA's for IGF-I and IGF-I receptor using the RNA'ase protection assay. Mice of ages 10 and 31 months have been treated daily for 5 or 10 days, killed, and tissues preserved. RNA extractions have been completed on muscle liver and kidney and quantification of specific mRNA's has begun. Total RNA (corrected for protein or tissue wet weight) and body weight were shown to increase in the treated mice, compared with age-matched placebo controls.

PROJECT NUMBER
Z01 AG 00021-29 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Normal Human Variability and Cross-cultural Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

C.C, Plato	Sr. Research Geneticist	LCP NIA
J.D. Tobin	Chief Applied Physiology	LCP NIA

COOPERATING UNITS (if any)
CNS NINDS; CPSB NCI; University of Maryland; Indiana University; University of Zagreb, Croatia.

LAB/BRANCH
Gerontology Research Center, Laboratory of Clinical Physiology

SECTION
Applied Physiology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.15	0.10

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects O (b) Human tissues O (c) Neither
O (a1) Minors
X (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project represents an ongoing collaborative effort, involving WHO and other national and international laboratories to coordinate the collection , evaluation and interpretation of normal genetic markers in order to study the cross-cultural patterns of genetic and extraneous factors as they relate to normative aging and to diseases with late onset, including Alzheimer's disease, breast cancer, Amyotrophic Lateral Sclerosis, and Parkinsonism Dementia. Specifically, the objectives of this study are: A) To study the cross cultural patterns of genetic and non-genetic factors in an effort to better understand the process of normative aging. B) To study the genetic segregation of these markers in families with late onset diseases, such as Alzheimer's disease, breast cancer, ALS and others, in an effort to establish genetic linkages and eventual identification of the factors responsible for these diseases. C) To study the distribution of dermatoglyphics, lateral dominance and other genetic variables in BLSA participants and other control samples, as well as in patients with late onset diseases.

PROJECT NUMBER

Z01 AG 00022-16 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Loss with Age: Epidemiological, Familial and Cross-Cultural Considerations.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

C.C. Plato Ph.D.	Sr. Research Geneticist	LCP NIA
J.D. Tobin M.D.	Chief, Applied Physiology	LCP NIA

COOPERATING UNITS (if any)

University Zagreb, Croatia; Nihon University, Tokyo; University of Maryland; Francis Scott Key Medical Ctr.; Johns Hopkins University; CNS, NINDS.

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	0.95	1.30

CHECK APPROPRIATE BOX(ES)

x (a) Human subjects ❑ (b) Human tissues ❑ (c) Neither

❑ (a1) Minors

❑ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the fourth decade of life, the human skeleton begins to lose bone. That is, bone mass decreases in relation to bone volume. Menopause and the associated estrogen deficiency will enhance bone loss in females. It has also been suspected that bone loss is familial, mainly because of the increased prevalence of osteoporosis in relatives, although there are no satisfactory scientific data to support either a familial or a genetic control of bone loss. In long bones, cortical bone is resorbed from the endosteal surface. Because of the thinning of the cortical bone shell, bones lose their mechanical integrity and fracture more readily. The trabecular bone mass of the vertebral column also decreases with age. The vertebral plates decrease in density, lose resistance to vertical compression stress and become more vulnerable to vertebral collapse. Vertebral compression fractures and fractures of the femoral neck are the most serious consequences of bone loss.

This project deals with the epidemiological, genetic, cross-sectional, longitudinal, and biomechanical aspects of bone loss (1) among the participants of the Baltimore Longitudinal Study ofAging (BLSA), (2) in genetic isolates of the Adriatic Sea Islands of Croatia and the island of Guam in Micronesia, (3) senior athlete population, (4) in long distance runners and relatively inactive normal controls, and (5) in rats and dogs.

PROJECT NUMBER
Z01 AG 00028-16 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Epidemiological and Genetic Studies of ALS Complex of Guam

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

C.C, Plato	Sr. Research Geneticist	LCP NIA
J.D. Tobin	Chief Applied Physiology	LCP NIA

OTHER INVESTIGATORS:

J. Bailey-Wilson	Louisiana State University
R.C. Elston	Louisiana State University

COOPERATING UNITS (if any)
R.M. Garruto, Supervisory Research Biologist CNS NINDS
D.C. Gajdusek, Chief, CNS NINDS

LAB/BRANCH
Gerontology Research Center, Laboratory of Clinical Physiology

SECTION
Applied Physiology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)

✱(a) Human subjects ❑ (b) Human tissues ❑ (c) Neither

❑ (a1) Minors

x (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Amyotrophic Lateral Sclerosis (ALS) and Parkinsonism dementia (PD) are neurological degenerative disorders that occur in three high incidence foci in the western Pacific: among the Chamorros of Guam and the northern Mariana islands, among the Japanese on the Kii peninsula and among the Auyu and Jakai peoples of West New Guinea. Clinically ALS and PD are two distinct disorders. However, preliminary analyses indicated that combining all three diagnoses (ALS, PD, and ALS+PD) into one affected diagnosis for genetic analyses, (thereby assuming any genetic effect on susceptibility to the two disorders was due to the same genetic mechanism) was reasonable. An Age, sex and birth cohort-specific liability was defined and segregation analysis was performed under both logistic and normal models for this liability at the time of disease onset. Under either model, purely environmental, Mendelian dominant and Mendelian recessive hypotheses could be rejected, but a two allele additive major locus hypothesis could not be rejected.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE Z01 AG 00290-06 LCP

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Osteoarthritis and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

J.D. Tobin	Chief, Applied Physiology Section	LCP NIA
C.C. Plato	Senior Research Geneticist	LCP NIA
Others:F. Wigley	Chief, Rheumatology	Francis Scott Key Med Center
W. Scott	Radiologist	Johns Hopkins Hospital
M. Hochberg	Rheumatologist	U. Maryland Medical School
M Lethbridge-Cejku	Research Associate	U. Maryland Medical School

COOPERATING UNITS (if any)

Frances Scott Key Medical Center (Wigley); Johns Hopkins Hospital (Scott), University of Maryland Med Sch (Lethbridge-Cejku and Hochberg)

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOXES)

| (a) Human subjects ❑ (b) Human tissues ❑ (c) Neither

❑ (a1) Minors

❑ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Osteoarthritis (OA) is the most common rheumatic disease of the elderly, with 40 million Americans estimated to have radiological evidence of OA. As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we have presented data on radiographic hand OA in men, and have evaluated the possible association of metabolic and physiologic factors with hand OA. We have now evaluated the prevalence of definite radiographic hand OA in 422 women of the BLSA, and have demonstrated that there is a marked increase in abnormal radiographs with age. Women under 40 years of age had < 1% abnormal readings which increased starting in the 40s with 25% abnormal, continuing in the 50's (77%) and 60's(94%) with >99% of the women over 70 having abnormal radiographs. In 325 women over the age of 40 estimates of fat mass and distribution were positively related to OA grade, while estimates of bone mass of the hand and arm were negatively related to OA grade. These associations were not present when the age distribution of the higher OA grades was accounted for. Similarly, serum levels of Insulin-like growth factor-I were inversely related to OA grade in 115 women aged 22-86, but this association also was not present when age was taken into account. These studies fail to support the hypothesis that physiologic and metabolic factors are associated with hand OA in women, independent of their known association with age.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE Z01AG 00293-04LCP

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Parameters of Bone Metabolism: Age and Sex Contrasts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affilliation.)

J.D. Tobin M.D.	Chief, Applied Physiology	LCP NIA

Other Investigators:

S.S. Sherman Ph.D.	Health Program Specialist	GP, NIA
C.C. Plato Ph.D.	Sr. Research Geneticist	LCP NIA
M.Gloth, MD	Guest Researcher	JHU

COOPERATING UNITS (if any)

Francis Scott Key Medical Center; Johns Hopkins University.

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.80	.40	.40

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects O (b) Human tissues O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Age-related changes in bone mass have been demonstrated in both men and women. Age and sex related differences in markers of bone turnover are important in elucidating changes in bone physiology in normal aging and disease. The relationship of 2 of these markers, Osteocalcin (OC) and urine pyridinoline (PYD), to age and sex and to each other was studied in 164 normal men and 101 normal women from the Baltimore Longitudinal Study of Aging. PYD increased linearly with age in both men and women ($p<.0001$), with mean PYD higher in females at all ages. The relationship of serum OC with age was biphasic, with levels in young men and women decreasing significantly while older men and women showed no significant correlation with age. Urine PYD levels were correlated to serum OC levels in both old men and old women but not in either young group. The relationship across the entire age range was significant after accounting for the effect of age by multiple regression.

Urinary PYD increases with age in both men and women, with women having higher levels across the entire age span. The relationship of PYD to OC in both men and women, independent of any effects of age, indicates coupling of these two markers of bone turnover. The lack of an association in the young groups may indicate a preferential process of bone accretion in the youngest adults.

Summary: Clinical Immunology Section LCP

The work in the Section continues to be directed to the exploration of cellular activation and the effects of an activation process. The findings have shown that the age related defect in T lymphocyte function is related to the choice of an activation pathway. Those pathways associated with the activation of the T cell antigen receptor and the synthesis of IL-2 show an age related decline in activity. However there are pathways either directly activating the PKc dependent chain of events or the CD2 non TCR related pathway which do not demonstrate an age related decline in activity. To investigate this finding we have instituted several approaches which include an intensive investigation of the normal chain of events that result in the induction of cellular function. To this end we have demonstrated a series of enzymes responsible for signal transduction through their ability to phosphorylate proteins. We have also detailed the effects of the cdc2 gene on T cell activity. Furthermore, although their is a decline in some activities for the T cells from an old host, there are actually increases in the synthesis of various lymphokines by these cells to levels far above that seen for cells from young donors. To understand this and to determine what effect this might have on cellular function in the old host we have instituted a project to detail the pattern of lymphokine synthesis both in vivo and in vitro by the T cells from old and young healthy and ill individuals. Also, we are investigating the expression of the genes for the lymphokines that are made at greater levels by the cells from old donors to determine the control and promotion of these genes in the cells. This investigation involves the study of DNA binding proteins and their role in gene expression. The work in the section will continue in these directions.

The projects on HIV infected individuals have demonstrated that their are complexes of HIV antigens, antibody and CD4 protein that circulate in the infected individual. These complexes are probably responsible for the blockage of the reticular endothelial phagocytic system described previously. An investigation of the immune response to the HIV continues and has shown changes in the response related to the stage of the illness. These projects will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00093-20-LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Basis or Regulation of the Humoral Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. A. Nordin	Research Chemist	LCP, NIA
Others:	Y. Kim	Visiting Fellow	LCP, NIA left 8/91
	M. A. Buchholz	Biologist	LCP, NIA
	F. J. Chrest	Biologist	LCP, NIA

COOPERATING UNITS *(If any)*

Drs. J. Shaper and N. Shaper, Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

LAB/BRANCH

Gerontology Research Center, Laboratory Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a2) Interviews

☐ (a1) Minors

SUMMARY OF WORK *(use standard unreduced type. Do not exceed the space provided.)*

The interaction of IL-2 with its receptor is critical not only to induce proliferation but is also required to allow activated T cells to complete the G1 phase of the cell cycle. The signal(s) generated through the IL-2/IL-2R interaction during the G1 phase upregulates the expression of certain critical T-cell activation genes and dictates the expression of cdc2. Disruption of the IL-2/IL-2R interaction blocks cells in late G1 and cell death occurs within 24 hours. The interaction of IL-2 with high affinity IL-2R that induces the entry of activated murine T-cells into S phase and initiates DNA synthesis is associated with an increase in the level of tyrosine phosphorylation of a single 97 kDa protein and a rapid transient increase in the kinase activity of p56lck.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00095-19-LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Cell Membrane Structures on Cellular Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. H. Adler	Medical Officer, PHS	LCP, NIA
Others:	J. E. Nagel	Medical Officer, PHS	LCP, NIA
	F. J. Chrest	Biologist	LCP, NIA
	S. M. Papciak	Staff Fellow	LCP, NIA EOD 12/91

COOPERATING UNITS (If any)

Drs. R. Winchurch and D. Kittur, Dept. Surgery, FSKMC, Johns Hopkins Univ., Dr. R. Chopra, School Hygiene, Johns Hopkins Univ., Baltimore, Maryland 21205

LAB/BRANCH

Gerontology Research Center, Laboratory Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.4	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a2) Interviews

☐ (a1) Minors

SUMMARY OF WORK (use standard unreduced type. Do not exceed the space provided.)

Although previous research on age-related changes in immune function have concentrated on the deficiency of factors that augment cellular function, it is now apparent that the levels of many lymphokines, cytokines, and inflammatory factors are elevated in serum and spleen cell culture supernatants from old mice. These factors, IL-6 TGFβ TNF and IFNγ can disrupt cellular function. The causes of the elevations are not known, but demonstrate that old mice and their cells are not deficient in their ability to make factors. In old rats, factors such as TGFβ and IL-6 may explain the differences seen in bone marrow cellular response to trauma, and IL-6 has been reported to prevent osteoporosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00096-19-LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Activation and Function in Aging Individuals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Brock	Research Biologist	LCP, NIA
Others:	W. H. Adler	Medical Officer, PHS	LCP, NIA
	F. J. Chrest	Biologist	LCP, NIA

COOPERATING UNITS (if any)

Dr. H. J. Hoffman, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD

LAB/BRANCH

Gerontology Research Center, Laboratory Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a2) Interviews

☐ (a1) Minors

SUMMARY OF WORK (use standard unreduced type. Do not exceed the space provided.)

Receptor mediated activation of many cell types is followed by motility related events. In T lymphocytes, lateral redistribution of surface receptors is accompanied by aggregation of actin and myosin in cytoplasmic subcaps and both are impaired in T cells from older individuals. Age-related changes both in basal levels of filamentous actin and in further polymerization of actin after activation of resting T cells from C57BL/6 mice with Concanavalin A were previously documented. Because of differences between the CD4 and CD8 positive subpopulations of T cells and technical problems using flow cytometry to document receptor mediated actin polymerization in mixed populations of the cells, a new procedure was developed to separate the subpopulations. Resting lymphocytes were isolated using discontinuous Percoll gradients. Superparamagnetic monodisperse polystyrene coated particles (Dynabeads) were coated with specific monoclonal antibodies to mouse T lymphocyte surface antigens. Coated beads were less efficient than T-cell recovery columns in removing B lymphocytes. Resting T cells were then incubated with CD4 or CD8 antibodies and coated beads using direct or indirect techniques. Negative selection of CD4 and CD8 positive cells in a magnetic field produced cells that were 98-100% viable, and contamination by the eliminated cell types was < 1.0%. These purified subpopulations from aged mice can now be used to characterize actin function, actin gene expression and kinetics of polymerization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00104-16-LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Immune Survey of Lognitudinal Project Participants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. H. Adler	Medical Officer, PHS	LCP, NIA
Others:	J. E. Nagel	Medical Officer, PhS	LCP, NIA
	L. Song	Visiting Fellow	LCP, NIA
	M. M. Schoonmaker	Biologist	LCP, NIA
	F. J. Chrest	Biologist	LCP, NIA
	G. D. Collins	Biologist	LCP, NIA
	R. S. Pyle	Biologist	LCP, NIA
	B. A. Dorsey-Cooper	Biologist	LCP, NIA

COOPERATING UNITS *(If any)*

None

LAB/BRANCH

Gerontology Research Center, Laboratory Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	1.7	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a2) Interviews

☐ (a1) Minors

SUMMARY OF WORK *(use standard unreduced type. Do not exceed the space provided.)*

These studies use participants in the Baltimore Longitudinal Study of Aging and human cell lines to gain insight into the biochemical and molecular mechanisms underlying age-assocciated changes in immune function. Recent data indicates that human T lymphocytes activated through different cellular pathways display distinct patterns of protein phosphorylation, cytokine synthesis and gene expression, only some of which are age-affected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00441-05-LCP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Host Factors Relating to HIV Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. H. Adler	Medical Officer, PHS	LCP, NIA
Others:	J. E. Nagel	Medical Officer, PHS	LCP, NIA
	G. D. Collins	Biologist	LCP, NIA
	M. Schoonmaker	Biologist	LCP, NIA
	R. S. Pyle	Biologist	LCP, NIA
	B. A. Dorsey-Cooper	Biologist	LCP, NIA

COOPERATING UNITS *(if any)*

Dr. Se-Kyung Oh, Dept. Biochemistry, Boston Univ.School of Medicine,
Dr. J. Bartlett, Dept. Medicine, Johns Hopkins Univ. Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATIONNIA, NIH, Balt

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a2) Interviews

☐ (a1) Minors

SUMMARY OF WORK *(use standard unreduced type. Do not exceed the space provided.)*

There are many factors which can influence the course of an HIV infection. The most powerful influence is the age of the infected person. Recent studies on the effects of age on immune function and on an HIV infection show that there are age related alterations in host responses with augmentation of the release of inflammatory factors. In an HIV infected individual there is an age related increase in the rate of helper T cell loss which may be due to a decline in the ability to replace HIV destroyed T cells.

LABORATORY OF MOLECULAR GENETICS

Overview:

The Laboratory of Molecular Genetics was established in 1985 in response to recommendations by the Board of Scientific Councilors and the National Advisory Council on Aging to establish such a facility within the intramural program of the National Institute on Aging to investigate the molecular basis for aging and age-dependent diseases.

The Laboratory of Molecular Genetics has had two major research foci, The Unit on Gene Expression and Aging and The Unit on Cell Proliferation and Aging. The Unit on Gene Expression and Aging is primarily concerned with genetic response to environmental toxins, stresses, oxygen radicals etc. Such systems, selected evolutionarily, result in the activation of specific genes that protect cells against damaging factors. The Unit on Cell Proliferation and Aging major focus is a novel antiproliferative protein, Prohibitin, identified based on the ability of its mRNA to block DNA synthesis when microinjected into cultured cells, including cancer cells. A new unit, The Unit on DNA Repair focuses on the mechanisms of DNA repair and their variations with differentiation, disease and aging. Most notably this group has pioneered in the investigation of DNA repair in specific genes. These studies have revealed striking differences in the repair of active and inactive genes, in the rate of repair by stem and differentiated cells, and in disease states. Many studies implicate DNA repair with aging and longevity.

Unit on Gene Expression and Aging

Heat Shock Protein Gene Expression in Response to Stress and Aging. Heat shock proteins (HSPs), a group of highly conserved proteins induced in response to a variety of cellular stresses, appear to be critical for maintaining cellular homeostasis. We have examined the expression of HSPs in mammalian cells following physiological stresses *in vivo* and *in vitro*.

In vivo studies: Previously we demonstrated that restraint or immobilization stress elicits the induction of HSP70 mRNA, the major HSP, in the adrenal gland and vasculature of rats. This stress-induced HSP70 expression was found to be attenuated with age. Over the past year we have further characterized this response, and have addressed the mechanism whereby restraint induces this response and the cause for its age-related decline. We have shown using Western analysis that induction of HSP70 mRNA by restraint is followed by an elevation in HSP70 protein. As seen for the mRNA, HSP70 protein expression is reduced in the aged animals. Induction of HSP70 in response to heat and other chemical stressors is mediated transcriptionally through the activation of a specific transcriptional factor, the heat shock factor (HSF), which binds to a specific DNA sequence,the heat shock element (HSE), in the promoter of HSP genes. We have shown that restraint results in the activation of HSF

in both the adrenal and vasculature tissues. Tissue extracts from aged animals show reduced levels of HSE-binding activity indicative of either reduced HSF activation or altered binding activity. These findings suggest that the differences in HSP70 expression observed between young and aged rats are likely to be due to differences in an early step in the signal transduction pathway leading to and/or involving transcriptional activation of the HSP70 gene.

In vitro studies: Prostaglandins (PG) of the A series are potent inhibitors of cell proliferation. We have shown that PGA_1 and PGA_2 induce HSP70 mRNA expression in mammalian cells. The effect was shown to be rapid, reversible, dose-dependent and specific for PG capable of arresting growth, and mediated through the activation of HSF. Induction was highly dependent on the growth state of cells, as induction occurred in growing, but not in confluent nongrowing cells. These results support a role for HSP70 in mediating the antiproliferative effects of PGAs.

gadd153, A Growth Arrest and DNA Damage Inducible Gene: Regulation by DNA Damage. *gadd153* is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It encodes a 19 kd protein with homology to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. This family consists of at least 5 members which contain a conserved bZIP carboxyl-terminal domain. The bZIP domain consists of a basic region involved in DNA recognition and binding, and an adjacent leucine zipper region, that mediates subunit dimerization. gadd153 is unique among C/EBP-related proteins in that it cannot bind to DNA. However, it can form stable heterodimers with C/EBP proteins and prevent their DNA-binding, suggesting that gadd153 acts as an inhibitor of these transcriptional activators. The functional significance of these interactions and their relationship to elevated *gadd153* expression following stress is unclear, but we have recently shown that two other C/EBP-related proteins, C/EBPß, and C/EBP‖, are also induced by DNA damage.

We have examined the role of calcium as a second messenger in regulating *gadd153* expression and have found that treatment of cells with the calcium ionophore A23187 leads to the rapid induction of *gadd153* mRNA. Elevated mRNA levels result from both an increase in the rate of *gadd153* transcription and an enhanced stability of *gadd153* mRNA. Buffering intracellular calcium by treatment with BAPTA-AM prevents *gadd153* induction by both A23187 and the DNA alkylating agent MMS. Thus, it is likely that intracellular calcium plays a role in controlling *gadd153* expression following DNA damage.

Inhibitors of various protein kinase and phosphatases have also been tested for their ability to influence *gadd153* expression following DNA damage. Protein kinase C inhibitors failed to inhibit *gadd153* mRNA induction as well as *gadd153* promoter activation by MMS. Tyrosine kinase inhibitors (genestein and vanadate) were also without effect. However, two general kinase inhibitors, 2-aminopurine and H7, both blocked *gadd153* mRNA expression. Okadaic acid and calyculin, specific inhibitors of phosphatases 1 and 2A, induced *gadd153* mRNA expression and enhanced *gadd153* promoter activity. Thus, *gadd153* appears to be under control of both protein kinase(s) and phosphatase(s), although the specific enzymes remain to be identified.

Previously a part of project Z01 AG 00713-04 LMG

Regulation of *gadd153* By Growth Arrest, Differentiation, and Metabolic Stresses. *gadd153* is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It encodes a 19 kd protein with homology to the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators. There is evidence to suggest that *gadd153* functions as a negative regulator of these positive transcriptional regulators, but the relationship between this purported function and its expression in response to growth arrest is unclear. In this project, studies have focused on determining mechanisms responsible for regulating *gadd153* expression in response to diverse growth inhibitory stimuli.

The cessation of growth which occurs when cells are grown to high density in the absence of medium replacement (high density medium depletion; HDMD) has been shown to result in the induction of *gadd153* mRNA. This induction is reversible and can be transferred to proliferating cells by the addition of medium from HDMD-arrested cells. Both transcriptional and posttranscriptional events are involved in controlling the response. Deletion analysis of the *gadd153* promoter region indicated that major elements necessary for transcriptional activation of the gene reside in a region between -778 and -250 relative to the transcriptional start site. Several consensus sequences corresponding to interleukin 6 (IL-6) response elements (IL-6RE) as well as a possible C/EBP binding site are present within this region. Preliminary evidence suggests that the IL-6RE sites play a role in mediating the response.

Glucose deprivation also leads to the induction of *gadd153* mRNA and contributes, at least in part, to the HDMD-mediated *gadd153* expression. However, additional mechanisms are involved. Nuclear run-on assays have shown that the effect of glucose deprivation is primarily at the transcriptional level, but surprisingly, glucose deprivation does not activate the same *gadd153* promoter fragment (-778 to +21) that is responsive to HDMD, suggesting that sequences outside of the 800 bp fragment tested are necessary for glucose-regulated expression of *gadd153*. .

Previously a part of project Z01 AG 00713-04 LMG

Genetic Response to Oxidative Damage. Oxidative stress, resulting from the exposure of cells to "reactive oxygen species" (ROS) is a major cause of both acute and chronic cell injury and is postulated to play an important role in aging. Major causes of oxidative stress in the cell include ionizing radiation, detoxification of foreign chemicals, inflammation, and normal metabolism. Intracellular targets of ROS include proteins, lipids and DNA. Although in bacteria many genes involving three distinct regulons have been shown to be induced by oxidative damage, little is known about the molecular response to oxidative stress in higher eukaryotes. Studies here have focused on characterization of the acute genetic response to oxidant damage in mammalian cells and tissues with hopes of identifying genes which play an important role in the cellular response to oxidative stress.

Three different *in vitro* model systems of oxidative stress have been employed including 1) hyperoxia (95% oxygen) treatment of cultured lung fibroblasts, 2) xanthine/xanthine oxidase treatment of rat proximal tubular epithelial cells, and 3) the effects of the nephrotoxic cysteine conjugate, S-(1,2-dichlorovinyl)-L-cysteine (DCVD) on porcine renal epithelial cells. *gadd153*, a CCAAT/enhancer-binding (C/EBP)-related gene and putative transcriptional regulator, was shown to be induced by each of the treatments, suggesting that it represents a generalized response to oxidant injury. In additional studies

with the *in vitro* cultured fibroblasts as well as *in vivo* studies examining lung tissue from rats exposed to 100% oxygen we have shown that two other C/EBP-related genes, C/EBPß and C/EBP‖, are also induced in response to hyperoxia.

We have begun studies to compare the sensitivity of aged versus young rats to the damaging effects of hyperoxia. We have observed that old rats (24 months of age) are less susceptible to hyperoxia-induced lung injury than are young (6 months of age) rats. Mortality resulting from respiratory distress in young rats placed in 100% oxygen occurred at an average exposure of 66 hours compared to 88 hours exposure in old rats. We are currently examining whether this age-related difference in survival is correlated with any age-related difference in the genetic response to hyperoxia.

Unit on Cell Proliferation and Aging

Cloning of a gene involved in shutting off cell growth. In earlier work, we identified a novel intracellular antiproliferative protein which we call prohibitin, and showed that prohibitin could block DNA synthesis in normal human fibroblasts and HeLa cells in vitro. In our most recent work, we have shown that prohibitin is highly conserved in evolution, expressed as a function of the stage of the cell cycle, and may play a role in processes as diverse as fruit fly development and human breast cancer.

Addendum
Unit on DNA Repair

GENE SPECIFIC DNA DAMAGE AND REPAIR

SUMMARY OF WORK:

We have established that there is intragenomic heterogeneity of DNA repair. Our findings have indicated that active genes are preferentially repaired in mammalian cells and that DNA repair efficiencies in specific genes are important for correlations to biological endpoints and to risk assessments. Whereas our earlier studies were limited to UV as a damaging agent, we have now developed methods to study DNA damage and repair after various carcinogens and cancer chemotherapeutic agents that react with DNA. A major research goal is to investigate the role of regional DNA repair in genomic instability which is a key aspect of malignancy and of the aging processes. We are studying the DNA repair of genes in a number of human, cancer prone DNA repair deficient syndromes and in various human and rodent mutant cell lines, some of which are transfected with repair genes. We are also investigating the role of DNA repair in multidrug resistance. Finally, we are now focusing increasingly on the role of DNA repair in cellular differentiation and aging.

OBJECTIVES:

1) To study DNA damage and repair in specific genes using established methodology. To develop new methodology to examine DNA damage and repair in structural genes

and oncogenes following treatment with various carcinogens, cancer chemotherapeutics, ionizing irradiation and oxidative damage.

2) To examine DNA repair of important genes in DNA damage sensitive, cancer prone or multidrug resistant human cell lines.

3) To investigate the biochemical and structural basis of gene specific repair.

4) To investigate the role of regional DNA repair in maintenance of genomic stability.

5) To characterize the role of DNA repair in longevity and aging.

Methodology: Cell culture techniques. DNA damage and repair. DNA molecular techniques, molecular hybridizations. RNA techniques. Hybridization and quantitation. General molecular biology. Centrifugation techniques. Densitometry. Plasmid analysis. Electroporetion. Computer programming. Drug pharmacology.

BRIEF SUMMARY OF PREVIOUS RESULTS.

We have demonstrated that essential genes in rodent and human cells undergo preferential repair after UV damage. In rodent cells, active genes are repaired much more efficiently than the bulk of the genome. In normal human cells, we have found that active genes are repaired faster than the bulk of the genome. It appears that determinations of DNA repair in specific genomic sequences may be more important than overall genome DNA repair measurements for correlations to other biological end points such as resistance to UV damage, transformation and cancer. Changes in preferential DNA repair could have profound effects on such parameters without noticeably altering overall genome repair levels since the vital regions only constitute a very small fraction of the genome.

We have found considerable differences in the repair efficiency of different genes within the same cell. The constitutively transcribed protooncogene c-abl is much more efficiently repaired than the transcriptionally silent proto-oncogene c-mos. In experiments measuring the repair in genes which can be modulated with regard to activity, results have further suggested that when metallothionein genes which are normally inactive become activated, there is a corresponding increase in DNA repair efficiency. These findings suggest a positive correlation between DNA repair efficiency and transcriptional activity in a gene. However, we have recently obtained results indicating that efficient gene specific DNA repair can be independent of transcription.

MAJOR FINDINGS

DNA damage and repair in genes after treatment with carcinogens, ionizing irradiation and chemotherapeutics.

With the use of the ABC excinuclease, we have developed methodology to quantitate cisplatinum intrastrand adducts, 4NQO, IQ (a highly mutagenic, heterocyclic compound) and NAAAF. We have been able to demonstrate preferential DNA repair of cisplatinum adducts in the CHO DHFR gene; NAAAF adducts were not preferentially repaired. In addition an assay has been developed to directly detect the sites of alkylation damage in DNA. In this assay, the DNA is nicked at alkylated sites by depurination and alkaline hydrolysis, and the frequency of alkylated sites in genes can then be measured. We have recently found that adducts formed after treatment with nitrogen mustard are initially formed at different frequencies in different genomic regions and that they are preferentially repaired in the CHO DHFR gene. Cisplatin intrastrand adducts can be quantitated within genes using the ABC excinuclease, and we have been able to detect cisplatin interstrand crosslinks using a denaturation-reannealing gel assay. Lately, we have been able to assay for X-ray induced single strand breaks in our hamster DHFR assay.

Studies of preferential DNA repair and strand specificity of DNA repair in the DHFR gene after treatment of CHO cells with UV and chemotherapeutics.

We have studied DNA damage and repair in the DHFR gene and in a noncoding region in CHO cells after different kinds of damage. CHO cells were UV irradiated or treated with nitrogen mustard, dimethyl sulphate or cisplatin. The DNA was extracted and after specific reactions, it was Southern blotted and probed. The frequency of pyrimidine dimers, 6-4 photoproducts, N7 alkylations, cisplatin intrastrand adducts and cisplatin interstrand crosslinks was determined in the different genomic regions. Pyrimidine dimers, 6-4 photoproducts, cisplatin intrastrand lesions and nitrogen mustard are preferentially repaired, i.e. there is significantly more efficient repair in the coding than in the noncoding region. In contrast, dimethylsulphate and cisplatin interstrand crosslinks are not preferentially repaired. The membranes were then reprobed with strand specific riboprobes for the DHFR gene to detect whether damage and repair was preferential in the coding strand. There are marked differences in the repair characteristics at the gene level for these different lesions. The most distinct strandspecificity (and the most distinct preferential repair) is seen for pyrimidine dimers. For the other lesions there is little or no strandspecificity. There may be a correlation between the extent of transcription blocking by a lesion and the strand specificity of its repair. Preferential DNA repair may be dependent upon the degree of chromatin structural change inflicted by a DNA damaging agent.

Repair of genes in human disease

We have analyzed DNA repair in specific genes in particularly cancer prone DNA damage sensitive or repair deficient syndromes including dysplastic naevus syndrome, Bloom syndrome, Cockaynes syndrome and Gardiner's syndrome. Preferential DNA repair after UV

damage was found in cells from patients with dysplastic nevus syndrome and Bloom syndrome, but it was deficient in Cockayne's syndrome and possibly in Gardiner's syndrome.

We have recently studied the repair of the essential DHFR gene in some xeroderma pigmentosum (XP) complementation groups and found that we could differentiate between 3 levels of DNA repair efficiency in the gene. Gene specific repair was determined in normal human cells, XP group C (XPC) (2 lines), XP group A (XPA) and XP group F (XPF). The relative levels of overall genome repair in these cells were: normal > XPC > XPF > XPA. The relative levels of UV resistance were: normal > XPF > XPC > XPA. In the analysis of repair of UV dimers in the gene, we found the relative efficiencies: normal > XPC > XPA > XPF. The XPF cell line is of interest since it has high survival, but low overall genome removal of pyrimidine dimers. We had expected a high level of gene repair in XPF, but found very little. This suggests that survival can be linked to the efficient repair of UV induced adducts other than the pyrimidine dimer. In XPA we found <u>some</u> repair of the DHFR gene; this is of interest since previous reports on UV repair in this cell line (only done at the level of the overall genome) shows no repair at all. Our findings thus suggest that there is repair activity in these repair deficient human cells, a novel preposition.

<u>Repair of mitochondrial DNA</u>

Mitochondrial DNA has been rather inaccessible to DNA repair studies since it very difficult to purify. The notion has prevailed, based on early studies of UV repair, that there is no DNA repair process in mitochondria. We have studied the mitochondrial repair in hamster cells by probing for damage in the entire mitochondrial genome. It appears that certain forms of DNA damage can be repaired from these sequences. Monofunctional alkylating agents and 4NQO are repaired efficiently in mitochondria while other lesions are not repaired. Evidently, these organelles possess repair enzymes, but their characterization awaits further study. Since mitochondria have no genes for these enzymes, they must be transported in.

MECHANISM OF PREFERENTIAL REPAIR

Effect on gene repair of inhibitors of topoisomerases and polymerases.
Various inhibitor experiments did not reveal a preferential involvement of any single, specific enzyme in the repair of an active gene. However, we find that when a topoisomerase I inhibitor (camptothecin) and a topoisomerase II inhibitor (merbarone) are added together, then the gene specific repair is strongly inhibited. Those studies suggest that topoisomerases <u>are</u> involved in gene specific repair, but that one type can take over for the other. We also find that caffeine inhibits the gene specific DNA repair. This may be via an effect on the cell cycle G1 arrest or it may be via the previously reported topisomerase inhibitory effect of caffeine.

Formation and repair of cisplatin lesions in cisplatin sensitive and resistant human ovarian cancer cell lines.
We have examined the formation and removal of cisplatin (CP) intrastrand adducts and inter-

strand crosslinks in specific genes (DHFR, MDR1, deltaglobin) in two CP-resistant human ovarian cancer cell lines. The intrastrand adducts were detected with the use of ABC excinuclease followed by Southern blot quantitation. The crosslinks were detected by a denaturation-renaturation procedure followed by gel-electrophoresis and Southern analysis. We found that cisplatin crosslinks were repaired much faster from specific genes in CP-resistant cell lines as compared to their parental CP-sensitive cell lines, while removal of cisplatin intrastrand adducts was similar in both the CP-resistant and the CP-sensitive cell lines. Since the rate of interstrand crosslink removal from the overall genome, as measured by alkaline elution, was similar in all cell lines, our findings suggest that DNA repair in specific genes may be important in the development of cellular resistance to chemotherapeutic agents.

DNA repair in the c-myc protooncogene in mouse B lymphocytes

We have examined DNA repair kinetics in selected regions of the c-myc and DHFR genes in normal B lymphoblasts derived from inbred mice that are resistant (DBA) or susceptible (BALB/c) to induced plasma cell tumors. DNA repair kinetics are similar in the protein-encoding portion of c-myc and in the DHFR gene in these cells. However, in the 5' flank of c-myc (a hotspot for translocation breakpoints in BALB/c plasmacytomas) BALB/c cells are repair deficient when compared to DBA cells. What is more, in the protein-encoding portion of c-myc, damage is selectively removed from only the transcribed strand. No repair is detected in the non-transcribed strand. In contrast, DNA repair in the 5' flank of c-myc is not strand specific; in DBA cells, damage is rapidly removed from both DNA strands, while in BALB/c cells no repair was detected in either strand in the 5' flank. These findings provide new insights into the details of gene-specific and strand-specific DNA repair and suggest that DNA repair and B cell neoplastic development may be closely associated.

DNA repair fine structure is a determinant for the distribution of mutations

We have recently made a direct comparison between the fine structure of DNA repair and the distribution of mutations. When hamster cells are treated with the heterocyclic amine benzophenanthrene diol epoxide, the spectrum of mutations in the DHFR gene are distributed with 92% in the nontranscribed strand and only 8% in the transcribed strand. The DNA repair under the same conditions, occurs solely in the transcribed strand. The indication is that the strand bias of DNA repair is the determinant for the distribution of mutations, and this implication may have widespread importance for our understanding of the mechanism of mutagenesis.

DNA repair in differentiated cell lines.

DNA repair was studied in selective genes that are either active or inactive in undifferentiated and terminally differentiated murine 3T3 cells. In the actively growing, undifferentiated cells, we found preferential DNA repair of active genes as compared to those genes that were not expressed. In the terminally differentiated cells, there was no difference between the repair levels in active and in inactive genes. The active genes were repaired with the same efficiency as the inactive genes in the undifferentiated cells. The implication here is that cells may lose the ability to preferentially repair their active genes in the cause of differentiation or aging.

PROPOSED COURSE

The objective is to further investigate aspects of preferential DNA repair in genes including its implication in malignancy, longevity and genomic instability.

A major characteristic of both cancer and senescence is the increased genomic instability. This is a vague term which includes chromosomal rearrangements, mutations, amplifications and translocations. In general, the underlying mechanism for these events are not understood. We have long speculated that local DNA repair processes may be important for the frequency of these events. Recently, we have found that the DNA repair efficiency is increased in the 5' flanking region of the c-myc protooncogene in mouse cells that are resistant to plasmacytoma. The indication is that the increased repair prevents the local translocations or other processes of importance. We have started to explore this in more detail by analyzing the repair in highly mutated genes such as the tumor suppressor gene p53, and by further analyzing the correlations between DNA repair fine structure strand selectivity and strand bias of mutations. We are also in the process of examining the repair characteristics in regions that are hotspots for translocations such as the pvt-1 locus in mouse cells. By looking at the repair in regions possessing different types of genomic instability, it is our ambitious hope that we can learn some general characteristics of the importance of DNA repair processes for the maintenance of the genomic integrity.

DNA repair characteristics will be examined in relation to senescence. This will be done at different levels: Gene specific repair of essential genes will be measured in young and old human fibroblasts, in cells from a patient with Werners syndrome, and in regions of the genome that are known to degerenerate with aging. One such region of particular biological interest is the telomeric structures where shortening with age has been observed. DNA repair has never been measured in these structures, and their location at the end of chromosomes may imply that the repair is different due to spatial or structural matters.

We are planning to investigate the gene-specific DNA repair characteristics in Drosophila melanogaster. This organism has well understood genetics and offers the select advantage that mutants with various kinds of neurological and age related deficiencies can be obtained.

Although most DNA damage and DNA repair studies are done using UV irradiation as the DNA damaging agent, a major source of DNA damage in humans is oxidative damage. It has been estimated, that in a 70 year old individual, 75% of the DNA would have been rendered nonfunctional due to inherent metabolic processes were it not for the DNA repair processes. Not much is understood about the DNA repair of oxidative damage, and no studies of this type of DNA repair are yet available at the level of individual genes. We are in the process of establishing the necessary techniques for such studies. Hydroxyguanosine and other oxidative lesions can be detected with specific endonucleases, and thus we should be able to create strand breaks at the site of the lesions. We then plan to study the damage and repair in individual genes using our already established techniques for UV. This should be of particular interest to investigate in mitochondrial DNA.

The strand bias of DNA repair (preferential repair of the transcribed strand) which has now

been observed appears to be due to a direct linkage between the DNA repair processes and transcription. Via work with an E. coli mutant lacking the ability to repair the transcribed DNA strand, a "transcription coupling factor" has been identified. This protein will become available for our use via an established collaboration with Dr. Sancar's laboratory where the factor was isolated. We then plan to microinject this factor into human cells that are repair deficient, such as the Cockayne's cells that lack the ability to repair active genes. Any characterization of enzymes involved in mammalian gene specific DNA repair should be very valuable. A further direction of this approach is to use various hamster mutant cells with transfected human repair genes coding for identified products.

Over the longer term it will most likely be necessary to establish an in vitro repair assay for the cellular and possibly for the gene specific DNA repair. This will enable us to better characterize the components involved in these processes.

RELATION TO INSTITUTE GOALS

A major hypothesis for senescence is based on the notion that DNA repair processes generally decline with age and that DNA damage gradually accumulates in the genome. This damage will then eventually inactivate individual genes and lead to a deterioration of the organism. Two types of experimental evidence have been invoked to support a role of DNA repair in the aging process. First, attempts have been made to correlate the life span of organisms with the overall genome efficiency of their DNA repair systems. In these studies, unscheduled DNA synthesis following UV irradiation has been used as a measure of the repair process. It was initially demonstrated by Hart and Setlow (1974) in a study of cells from several mammalian species that there was a linear relationship between the logarithm of the lifespan and the unscheduled DNA synthesis in its cells. Subsequent studies with additional mammalian species and with additional classes of organisms have not universally supported this trend, however. The second approach has been to look for an attenuation of the DNA repair efficiency in cells from aging individuals or in senescent cultures. The results from many such studies have provided no clear consensus for a correlation between overall genome DNA repair and the aging processes. However, these previous studies have not taken into consideration the recently established heterogeneity of DNA repair processes. In studies of DNA repair in the total genome, major changes in selective, important genes would not be detectable since the genes only constitute about 1% of the total cellular DNA.

We have recently reported that in a murine proadipocyte differentiation system there is a loss of preferential DNA repair of active genes with terminal differentiation (Bill et al., J. Biol. Chem. 266, 21821-21826, 1991). This suggests that whereas the relationship between overall genome DNA repair and the aging process is not evident, such a relationship may exist when the repair process is determined at the gene level. These provoking data justify further exploration as mentioned above.

Relation to ongoing studies in LMG, NIA

A major focus of ongoing research in the Laboratory of Molecular Genetics is the investi-

gation of the damage inducible responses and their modulation with senescence. It is not clear whether any of the damage inducible genes in mammalian systems are repair enzymes, and this question will be of general interest in the Laboratory. The common denominator for the research in the Laboratory will be responses to DNA damage and their relation to the aging process.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00705-07 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning of a gene involved in shutting off cell growth.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D.B. Danner	Senior Research Investigator	LMG, NIA
Others:			
	D.A. Stewart	Biologist	LMG, NIA
	C.M. Wood	NRC Fellow	LMG, NIA
	G.A. Owens	Biologist	LMG, NIA
	A.J.I. Roskams	IRTA Fellow	LMG, NIA
	M.A. Altus	IRTA Fellow	LMG, NIA

COOPERATING UNITS *(if any)*

Noble Foundation, Ardmore, OK; Pathology Dept., Harvard Med. School; University of Utah; Developmental Biol. Center, U.C. Irvine; Human Genetics Dept. Roswell Park, NY; Institute for Molecular Genetics, Baylor; Biochem. Dept., Cancer Inst., Tokyo

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.6	5.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues x

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In earlier work, we identified a novel intracellular antiproliferative protein which we call prohibitin, and showed that prohibitin could block DNA synthesis in normal human fibroblasts and HeLa cells *in vitro*. In our most recent work, we have shown that prohibitin is highly conserved in evolution, expressed as a function of the stage of the cell cycle, and may play a role in processes as diverse fruit fly development and human breast cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG00710-04 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heat Shock Protein Gene Expression in Response to Stress and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Nikki J. Holbrook	Senior Investigator	LMG, NIA
Others:			
	James D. Putney	Staff Fellow	LMG, NIA
	Sara G. Carlson	Biologist	LMG, NIA

COOPERATING UNITS (if any)

Department of Surgery, The Johns Hopkins University and Hospital (R. Udelsman)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Heat shock proteins (HSPs), a group of highly conserved proteins induced in response to a variety of cellular stresses, appear to be critical for maintaining cellular homeostasis. We have examined the expression of HSPs in mammalian cells following physiological stresses *in vivo* and *in vitro*.

In vivo studies: Previously we demonstrated that restraint or immobilization stress elicits the induction of HSP70 mRNA, the major HSP, in the adrenal gland and vasculature of rats. This stress-induced HSP70 expression was found to be attenuated with age. Over the past year we have further characterized this response, and have addressed the mechanism whereby restraint induces this response and the cause for its age-related decline. We have shown using Western analysis that induction of HSP70 mRNA by restraint is followed by an elevation in HSP70 protein. As seen for the mRNA, HSP70 protein expression is reduced in the aged animals. Induction of HSP70 in response to heat and other chemical stressors is mediated transcriptionally through the activation of a specific transcriptional factor, the heat shock factor (HSF), which binds to a specific DNA sequence,the heat shock element (HSE), in the promoter of HSP genes. We have shown that restraint results in the activation of HSF in both the adrenal and vasculature tissues. Tissue extracts from aged animals show reduced levels of HSE-binding activity indicative of either reduced HSF activation or altered binding activity. These findings suggest that the differences in HSP70 expression observed between young and aged rats are likely to be due to differences in an early step in the signal transduction pathway leading to and/or involving transcriptional activation of the HSP70 gene.

In vitro studies: Prostaglandins (PG) of the A series are potent inhibitors of cell proliferation. We have shown that PGA_1 and PGA_2 induce HSP70 mRNA expression in mammalian cells. The effect was shown to be rapid, reversible, dose-dependent and specific for PG capable of arresting growth, and mediated through the activation of HSF. Induction was highly dependent on the growth state of cells, as induction occurred in growing, but not in confluent nongrowing cells. These results support a role for HSP70 in mediating the antiproliferative effects of PGAs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00720-03 LMG

PERIOD COVERED

October 1, 1991 to July 5, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression in Chronic Wounds and Aged Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	George R. Martin	Scientific Director	NIA
Others:			
	Elizabeth M. Burke	Staff Fellow	LMG, NIA
	Walter E. Horton	Senior Investigator	LMG, NIA
	Michael T. Crow	Staff Fellow	LMG, NIA
	Jay D. Pearson	Staff Fellow	LMG, NIA

COOPERATING UNITS *(if any)*

Department of Geriatrics, Johns Hopkins School of Medicine (W.B. Greenough, R. B. Bennett; Department of Pathology, Johns Hopkins School of Medicine (M. Taylor)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, Baltimore, Maryland 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aberrant wound healing is a common problem in both the ambulatory and bedbound geriatric population. We have been studying wound healing in both in vitro and in vivo systems in an attempt to understand how the process may be altered in aging.

We have demonstrated an increase in the interstitial collagenase mRNA, an increased ratio of interstitial collagenase to collagen mRNA, and an increased ratio of interstitial collagenase to TIMP-1 (tissue inhibitor of metalloproteinases) mRNA in fibroblasts cultured from healthy older donors compared with healthy young donors both at basal levels and after treatment with a tumor promoting agent. These studies are consistent with observation made on cultured cells which show an overproduction of collagenase in senescent cells. To some extent, the cells cultured from the skin of older individuals show similar but lesser changes.

In vivo we have characterized the expression of interstitial collagenase, 92 kDa type IV collagenase, 72 kDa type IV collagenase, the tissue inhibitors of metalloproteinases types 1 and 2, and pro-α2 (I) collagen in chronic decubiti and normal skin from the same donors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00721-02 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Response to Oxidative Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Nikki J. Holbrook	Senior Investigator	LMG, NIA
	Augustine M.K. Choi	Guest Researcher	LMG, NIA
	Yusen Liu	Visiting Fellow	LMG, NIA

COOPERATING UNITS (if any)

Department of Pathology, Univ. Maryland School of Medicine (Dr. Benjamin F. Trump)
W. Alton Jones Cell Science Center, Lake Placid, NY (Dr. James L. Stevens)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oxidative stress, resulting from the exposure of cells to "reactive oxygen species" (ROS) is a major cause of both acute and chronic cell injury and is postulated to play an important role in aging. Major causes of oxidative stress in the cell include ionizing radiation, detoxification of foreign chemicals, inflammation, and normal metabolism. Intracellular targets of ROS include proteins, lipids and DNA. Although in bacteria many genes involving three distinct regulons have been shown to be induced by oxidative damage, little is known about the molecular response to oxidative stress in higher eukaryotes. Studies here have focused on characterization of the acute genetic response to oxidant damage in mammalian cells and tissues with hopes of identifying genes which play an important role in the cellular response to oxidative stress.

Three different *in vitro* model systems of oxidative stress have been employed including 1) hyperoxia (95% oxygen) treatment of cultured lung fibroblasts, 2) xanthine-xanthine oxidase treatment of rat proximal tubular epithelial cells, and 3) the effects of the nephrotoxic cysteine conjugate, S-(1,2-dichlorovinyl)-L-cysteine (DCVD) on porcine renal epithelial cells. *gadd153*, a CCAAT/enhancer-binding (C/EBP)-related gene and putative transcriptional regulator, was shown to be induced by each of the treatments, suggesting that it represents a generalized response to oxidant injury. In additional studies with the *in vitro* cultured fibroblasts as well as *in vivo* studies examining lung tissue from rats exposed to 100% oxygen we have shown that two other C/EBP-related genes, C/EBPß and C/EBP-, are also induced in response to hyperoxia.

We have begun studies to compare the sensitivity of aged versus young rats to the damaging effects of hyperoxia. We have observed that old rats (24 months of age) are less susceptible to hyperoxia-induced lung injury than are young (6 months of age) rats. Mortality resulting from respiratory distress in young rats placed in 100% oxygen occurred at an average exposure of 66 hours compared to 88 hours exposure in old rats. We are currently examining whether this age-related difference in survival is correlated with any age-related difference in the genetic response to hyperoxia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00722-01 LMG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of *gadd135* By Growth Arrest, Differentiation, and Metabolic Stresses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Nikki J. Holbrook	Senior Investigator	LMG, NIA
Other:			
	Sara G. Carlson	Biologist	LMG, NIA
	Colette ap Rhys	IRTA Fellow	LMG, NIA
	Sherrie Reichenbaugh	Biologist	LMG, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.2	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

gadd153 is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It encodes a 19 kd protein with homology to the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators. There is evidence to suggest that *gadd153* functions as a negative regulator of these positive transcriptional regulators, but the relationship between this purported function and its expression in response to growth arrest is unclear. In this project, studies have focused on determining mechanisms responsible for regulating *gadd153* expression in response to diverse growth inhibitory stimuli.

The cessation of growth which occurs when cells are grown to high density in the absence of medium replacement (high density medium depletion; HDMD) has been shown to result in the induction of *gadd153* mRNA. This induction is reversible and can be transferred to proliferating cells by the addition of medium from HDMD-arrested cells. Both transcriptional and posttranscriptional events are involved in controlling the response. Deletion analysis of the *gadd153* promoter region indicated that major elements necessary for transcriptional activation of the gene reside in a region between -778 and -250 relative to the transcriptional start site. Several consensus sequences corresponding to interleukin 6 (IL-6) response elements (IL-6RE) as well as a possible C/EBP binding site are present within this region. Preliminary evidence suggests that the IL-6RE sites play a role in mediating the response.

Glucose deprivation also leads to the induction of *gadd153* mRNA and contributes, at least in part, to the HDMD-mediated *gadd153* expression. However, additional mechanisms are involved. Nuclear run-on assays have shown that the effect of glucose deprivation is primarily at the transcriptional level, but surprisingly, glucose deprivation does not activate the same *gadd153* promoter fragment (-778 to +21) that is responsive to HDMD, suggesting that sequences outside of the 800 bp fragment tested are necessary for glucose-regulated expression of *gadd153*. .

Previously a part of project Z01 AG 00713-04 LMG

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00723-01 LMG

PERIOD COVERED

October 1, 1991 to September 1, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

gadd153, A Growth Arrest and DNA Damage Inducible Gene: Regulation by DNA Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Nikki J. Holbrook	Senior Investigator	LMG, NIA
Others:			
	Jong Sung Park	Visiting Associate	LMG, NIA
	Jennifer D. Luethy	Biologist	LMG, NIA
	Sara G. Carlson	Biologist	LMG, NIA
	John D. Bartlett	IRTA Fellow	LMG, NIA
	Augustine M.C. Choi	Guest Researcher	LMG, NIA
	Tim Fawcett	Staff Fellow	LMG, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

gadd153 is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It encodes a 19 kd protein with homology to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. This family consists of at least 5 members which contain a conserved bZIP carboxyl-terminal domain. The bZIP domain consists of a basic region involved in DNA recognition and binding, and an adjacent leucine zipper region, that mediates subunit dimerization. gadd153 is unique among C/EBP-related proteins in that it cannot bind to DNA. However, it can form stable heterodimers with C/EBP proteins and prevent their DNA-binding, suggesting that gadd153 acts as an inhibitor of these transcriptional activators. The functional significance of these interactions and their relationship to elevated *gadd153* expression following stress is unclear, but we have recently shown that two other C/EBP-related proteins, C/EBPβ, and C/EBP-‖, are also induced by DNA damage.

We have examined the role of calcium as a second messenger in regulating *gadd153* expression and have found that treatment of cells with the calcium ionophore A23187 leads to the rapid induction of *gadd153* mRNA. Elevated mRNA levels result from both an increase in the rate of *gadd153* transcription and an enhanced stability of *gadd153* mRNA. Buffering intracellular calcium by treatment with BAPTA-AM prevents *gadd153* induction by both A23187 and the DNA alkylating agent MMS. Thus, it is likely that intracellular calcium plays a role in controlling *gadd153* expression following DNA damage.

Inhibitors of various protein kinase and phosphatases have also been tested for their ability to influence *gadd153* expression following DNA damage. Protein kinase C inhibitors failed to inhibit *gadd153* mRNA induction as well as *gadd153* promoter activation by MMS. Tyrosine kinase inhibitors (genestein and vanadate) were also without effect. However, two general kinase inhibitors, 2-aminopurine and H7, both blocked *gadd153* mRNA expression. Okadaic acid and calyculin, specific inhibitors of phosphatases 1 and 2A, induced *gadd153* mRNA expression and enhanced *gadd153* promoter activity. Thus, *gadd153* appears to be under control of both protein kinase(s) and phosphatase(s), although the specific enzymes remain to be identified.

Previously a part of project Z01 AG 00713-04 LMG

ANNUAL REPORT OF THE
LABORATORY OF PERSONALITY AND COGNITION
NATIONAL INSTITUTE ON AGING
1991-1992

OVERVIEW

The fundamental scientific paradigm which guides research in the Laboratory of Personality and Cognition (LPC) is the analysis of individual differences. Few phenomena are more basic than the fact that human beings differ—in health, in rates of aging, in cognitive ability, in personality, in happiness and life satisfaction. The mission of the LPC is threefold: (1) to conduct basic and clinical research on individual differences in cognitive and personality processes and traits; (2) to investigate the influence of age on these variables and their reciprocal influence on health, well-being, and adaptation; and (3) to employ longitudinal, experimental, and epidemiological methods in the analysis of psychological and psychosocial issues of aging, including health and illness, predictors of intellectual competence and decline, models of adult personality, and correlates of disease risk factors.

COGNITION AND NEUROPSYCHOLOGY

During the past year, the Chief of the Cognition Section, Dr. Herbert Weingartner returned to NIAAA to assume full-time responsibilities directing research on psychopharmacology and cognition. In his place, we recruited a Senior Staff Fellow, Dr. Susan M. Resnick from the University of Pennsylvania. Dr. Resnick, an experimental neuropsychologist with training in behavioral genetics, cognitive assessment, and neuroimaging has worked with the MRI and PET groups at the University of Pennsylvania. She has done extensive research in brain-behavior relations in psychiatric populations.

Dr. Resnick will bring her skills and experiences to an important new long-term study of changes in brain structure and function using neuroimaging procedures. This project will perform annual neuroimaging studies on 150 selected BLSA participants aged 60 years and older over a 9-year period. State-of-the-art magnetic resonance imaging (MRI) and positron emission tomography (PET) will be used to measure brain anatomy and blood flow, respectively. PET and oxygen-15 labeled water will provide measurements of cerebral blood flow during resting conditions and during the performance of cognitive tasks. A battery of

neuropsychological tests will be administered in conjunction with the neuroimaging evaluations. This project will provide important information on the relation between longitudinal changes in neuropsychologic performance and changes in regional brain structure and activity in aging men and women. Moreover, the extensive amount of prior physical and psychological data available for the BLSA participants will enhance our understanding of predictors of individual differences in the rate of neuroanatomic, neurophysiologic and cognitive aging. The neuroimaging data will additionally serve as a normative base from which to evaluate pathological aging in the elderly.

As part of this neuroimaging effort, Drs. Giambra and Zonderman participated in training programs in the use and interpretation of magnetic resonance imaging, positive emission tomography, and single photon emission computerized tomography at The Johns Hopkins Medical Institutions and at the National Institutes of Aging.

This year major progress has been achieved in revising the cognitive testing program to focus on tasks that show promising predictions of cognitive impairment. With this in mind, the memory and reaction time study was reformulated. Tasks that were not related or predictive of cognitive impairment were dropped, and tasks assessing long-term memory were added.

Significant progress has also been made in our Early Markers of Alzheimer's Disease (EMAD) program. Participants in the Baltimore Longitudinal Study of Aging aged 60 and older were examined to detect changes in psychological, neurological, and neuropsychological tests related to early signs of Alzheimer's disease. Mental status tests are often used to screen and evaluate individuals for significant cognitive impairment or dementia. Research completed this year has demonstrated that individual differences in cognitive performance on the Mini-Mental State Examination, one of the most widely used dementia screening instruments, can be predicted up to 9-15 years later by performance on a nonverbal test of memory, the Benton Visual Retention test (BVRT). Extensive statistical analyses revealed that the predictive power of BVRT scores was independent of the effects of age and intelligence which are associated with BVRT performance. In addition to the BVRT, performance on tests of immediate and short-term delayed memory tasks for words and numbers, along with scores from several continuous performance tasks also significantly predicted mental status among women and men in the BLSA. Ongoing research will attempt to determine whether the BVRT and MRT scores are correlates of incipient cognitive impairment or are truly forecasting subsequent changes in mental impairment.

BASIC RESEARCH ON PERSONALITY STRUCTURE

The Five-Factor Model of personality has become the cornerstone of research for the Personality, Stress and Coping Section (PSCS). The five dimensions of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness form a comprehensive framework for conceptualizing individual differences in basic emotional, interpersonal, experiential, attitudinal, and motivational styles. In the past year, investigators have continued to refine their conceptions of the factors and broadened their applications.

One line of research concerned the heritability of the five factors. There have been dozens of studies on the heritability of Neuroticism and Extraversion, but very few on Openness, Agreeableness, or Conscientiousness. Data collected several years ago by colleagues working with a sample of Swedish twins were recently analyzed; they showed moderate heritability for measures of Openness and Conscientiousness, but no evidence of heritability for Agreeableness. This finding stands in contrast to some studies that have reported substantial heritability for aggression and other traits related to low Agreeableness, and poses important new questions for studies of behavior genetics.

The Five-Factor Model has been adopted by a number of researchers as a comprehensive model of personality. As such, it can systematize research in many applied areas. A recent Special Issue of the *Journal of Personality*, edited by PSCS Research Psychologist Dr. Robert R. McCrae, surveyed theoretical issues in the Five-Factor Model as well as applications to such areas as moods and emotions, interpersonal transactions, and health psychology. The model is also featured in a symposium on personality assessment held at the American Psychological Association Convention and chaired by Dr. McCrae.

Clinical Applications of Personality Research

Problems in mental health and adjustment—from depression and anxiety to marital and sexual difficulties—are faced by adults of all ages. One of the important applications of LPC research on personality, stress, and coping is in the guidance it can offer to counselors, clinical psychologists, and psychiatrists. This year Laboratory investigators continued to disseminate their findings to these practitioners.

Depression in the elderly is a topic of growing importance and interest, and was the focus of an NIH Consensus Development Conference on the Diagnosis and Treatment of Depression in Late Life. LPC researchers presented their research on depression as an

enduring disposition to this conference, making the point that there is often no clear boundary between clinical depression and normal variations in the experience of sadness and hopelessness. Longitudinal findings on BLSA subjects thus have relevance to the understanding and treatment of clinical depression.

Research from this Laboratory is also directly relevant to another group of psychiatric conditions—the personality disorders. Earlier research showed links between measures of the Five-Factor Model and scales intended to assess personality disorders. This line of research has been pursued by many other researchers here and abroad, and is the subject of a book entitled *Personality Disorders and the Five-Factor Model of Personality*, co-edited by the Chief, LPC, and published by the American Psychological Association. The book includes research on clinical populations and provides suggestions for the use of measures of normal personality in diagnosing and treating personality disorders.

Finally, the Chief, LPC, has been invited to participate in a Symposium on "Clinical Personality Assessment in the 1990s" presented at the American Psychological Association. He will describe the recent revision of the NEO Personality Inventory and discuss its use in clinical settings.

Aging, Stress, and Coping

LPC researchers have continued to contribute to advances in our understanding of stress and coping. The Chief, LPC, has been invited to co-chair a symposium on "Stress and Coping" at the XVth International Congress of Gerontology in Budapest, and will present a paper there on generality and specificity in stress and coping processes in old age.

Much of the research of this Laboratory has been devoted to an examination of age changes in personality, and to the documentation of stability of personality traits across the adult lifespan. The evidence on stability conflicts with many theories of adult development and with widely held ideas on personality changes thought to be brought about by psychotherapy. In an attempt to resolve these issues, the American Psychological Association's Science Weekend devoted one full day of programming to the theme, "Can Personality Change?", including a presentation by the Chief, LPC. A book based on these presentations will be published by APA.

PROJECT NUMBER Z01 AG 00180-07 LPC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Stress, Coping and Personality in Aging Men and Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Robert R. McCrae	Research Psychologist	LPC, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)
Longitudinal Studies Branch

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center,
Baltimore, MD 21224

TOTAL MAN-YEARS: 2.1 PROFESSIONAL: 1.1 OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure of personality traits in late adolescence and age differences between age 17 and age 64 were examined using data from two samples. Both college students (N = 389) and adults (N = 1,539) completed the Revised NEO Personality Inventory. The factor structure of the inventory was essentially the same in the college group as in normative adult samples. Cross-sectional comparisons suggested that adults are somewhat less emotional and somewhat more socialized than college students, and the adult personality development continues during the decade of the 20s. Longitudinal research on personality, stress and coping will continue.

PROJECT NUMBER Z01 AG 00183-04 LPC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Basic Research in Personality

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center,
Baltimore, MD 21224

TOTAL MAN-YEARS: 1.3 PROFESSIONAL: 1.1 OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Personality can be defined in terms of enduring individual differences in emotional, interpersonal, experiential, and motivational styles. The five factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness provide a comprehensive taxonomy of personality traits for the description of personality in aging men and women. Two studies were conducted as part of continuing program of research on these factors. In the first, alternative measures of the five factors were compared in peer ratings and both general agreement and specific differences were found. In the second, the correlation between general intelligence and Openness to Experience was examined, and showed that, although related, Openness is not equivalent to intelligence, and may be more strongly related to creativity.

PROJECT NUMBER Z01 AG 00184-04 LPC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Psychosocial Predictors of Mental and Physical Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA
Stephanie V. Stone	Staff Fellow	LPC, NIA
Chester A. Schmidt	Special Volunteer	FSKMC

COOPERATING UNITS (if any)
Psychiatry Department, FSKMC

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center,
Baltimore, MD 21224

TOTAL MAN-YEARS: 2.8 PROFESSIONAL: 1.6 OTHER: 1.2

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The associations among Neuroticism, self-rated health, and mortality were examined in a national sample of 1,472 women initially aged 45 to 64 and followed over a 16 year period. Self-rated health was related to Neuroticism, which was unrelated to mortality. However, self-rated health was a significant prospective risk factor for all-cause mortality, net of other risk factors. Efforts to relate basic research on personality to applications in clinical psychology and studies of the relation of personality to physical health will continue.

PROJECT NUMBER Z01 AG 00185-04 LPC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Early Markers of Alzheimer's Disease in Longitudinal Participants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Alan B. Zonderman	Research Psychologist	LPC, NIA
Leonard M. Giambra	Research Psychologist	LPC, NIA
Susan Resnick	Senior Staff Fellow	LPC, NIA
Claudia H. Kawas	Staff Neurologist	FSKMC
Robert R. McCrae	Research Psychologist	LPC, NIA
E. Jeffery Metter	Medical Officer	LSB, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA

COOPERATING UNITS (if any)
Longitudinal Studies Branch
Department of Neurology, FSKMC

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center,
Baltimore, MD 21224

TOTAL MAN-YEARS: 4.8 PROFESSIONAL: 1.8 OTHER: 3.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Participants in the Baltimore Longitudinal Study of Aging aged 60 and older were examined to detect changes in psychological, neurological, and neuropsychological tests related to early signs of Alzheimer's disease. After controlling for age and WAIS Vocabulary, the Benton Visual Retention (BVR) test accounted for 5% of the variance in subsequent scores on the cognitive impairment assessed by the Mini-Mental Status Examination (MMSE) 6-15 years later. The correlations between the BVR and MMSE over 6-8 and 9-15 years were .36 and .34 ($p < .05$). In addition, a battery of memory and reaction time tests consisting of five continuous performance tasks and tests of immediate and short-delayed memory tasks for words and numbers (MRT) accounted for 14% of MMSE variance 6-8 years later. These results provide preliminary evidence that mental status can be predicted, at least in part, by earlier performance on cognitive tests.

PROJECT NUMBER Z01 AG 00189-02 LPC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Attentional Processes in Normal and Impaired Elderly

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)
Leonard M. Giambra Research Psychologist LPC, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Cognition

INSTITUTE AND LOCATION National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224

TOTAL MAN-YEARS: 1.0 PROFESSIONAL: 0.50 OTHER: 0.50

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We seek to understand the psychological and biopsychological aspects of normal and pathological aging in terms of attention and attentional processes. We are also concerned with applying that knowledge to develop strategies for improving attentional and cognitive functioning. This year we report on longitudinal changes in spontaneous shifts of attention from external stimuli to the contents of consciousness derived from retrospective reports taken six to eight years apart. In a sample of 93 women and 169 men, initially 24-71 years old, we found for all ages equivalent decreases in the likelihood of spontaneous shifts. These results are consistent with cross-sectional age differences reported previously. Spontaneous shifts may require that there be attractive thoughts in consciousness. Fewer thoughts breaking into consciousness may provide fewer opportunities for spontaneous shifts of attention to them. We have previously reported that involuntary attentional shifts to internal stimuli were more frequent in adults who were hyperactive as children and less frequent with deeper levels of depression. Together, these outcomes are consistent with the view that involuntary shifts of attention to internal mental events may be driven by the level of thought production in the brain.

ANNUAL REPORT OF THE LABORATORY OF NEUROSCIENCES
NATIONAL INSTITUTE ON AGING
1991-1992

I. ORGANIZATION AND MISSION STATEMENTS

The Laboratory of Neurosciences (LN) at the National Institute on Aging was formed in 1978, and is involved in research on the central and peripheral nervous systems in health, aging and disease, including Alzheimer's disease. The Laboratory is located at the Clinical Center in Bethesda, Maryland, and is divided into three sections entitled, (1) Cerebral Physiology and Metabolism, (2) Brain Aging and Dementia, and (3) Neurochemistry and Brain Transport. In addition, there are four Units which were formed in 1987 and 1988, entitled (1) Positron Emission Tomography, (2) Neuropsychology, (3) Pharmacology and Pharmacokinetics, and (4) Brain Imaging and Computers. In September 1982, a six-bed temporary Patient Care Unit (PCU) was established to study inpatients with Alzheimer's disease and other dementias, as well as healthy subjects. The PCU, moved to permanent quarters on the 6D Ward in 1990. An Outpatient Clinic also was started in 1982 to screen subjects of inpatient protocols and to carry on outpatient-related research.

A. SECTION ON CEREBRAL PHYSIOLOGY AND METABOLISM (STANLEY I. RAPOPORT, CHIEF)

This section investigates the function, structure, physiology, biochemistry, and pharmacology of the central and peripheral nervous systems and the changes that take place during development and aging. Areas of investigation include the application of in vivo techniques to study brain glucose and lipid metabolism (using radioactive 2-deoxy-D-glucose or fatty acids); examination of the structure and function of the blood-brain and blood-nerve barriers, in disease models and before and following modification, in relation to chemotherapy of central nervous system disease; the use of tissue culture techniques to examine neuronal electrical properties in relation to altered genetic composition (trisomy 16 mice); the use of histological techniques to examine neuronal morphology and plasticity; neurochemical and molecular biological techniques to examine the Alzheimer brain.

B. SECTION ON BRAIN AGING AND DEMENTIA (MARK B. SCHAPIRO, CHIEF)

This section examines the metabolic, anatomical, neurochemical and neuropsychological parameters that characterize cerebral function in the following subject groups, so as to understand aging and disease of the brain and to provide a differential diagnosis of Alzheimer's disease and other dementias: (1) healthy men and women at different ages; (2) dementia of the Alzheimer type; (3) Down syndrome; (4) multiple infarct dementia; (5) treated chronic hypertensives; (6) depression in the elderly. The section employs positron emission tomography to examine cerebral metabolic rates for glucose and cerebral blood flow, computerized CT and magnetic resonance imaging to evaluate brain anatomy magnetic resonance spectroscopy to evaluate brain metabolism, analytical techniques to explore the composition of cerebrospinal fluid, and neuropsychological tests to evaluate the details of cognitive function. The program has initial cross-sectional studies followed by longitudinal studies with post-mortem follow up.

C. UNIT ON POSITRON EMISSION TOMOGRAPHY (CHERYL L. GRADY, CHIEF)

This unit is responsible for developing and conducting research involving positron emission tomography on human subjects in relation to aging, dementia, including Alzheimer's disease and multiple infarct dementia (see Section heading), and developmental abnormalities of the brain, including retardation.

Clinical protocols are formulated to examine brain glucose utilization using 18-F-2-deoxy-D-glucose, and blood flow using 150-water, as positron-emitting tracers. Studies are performed on subjects at rest, with reduced visual and auditory inputs, and under conditions of well-defined cognitive or physiological stimulus paradigms. Metabolic and flow data are related to data obtained with CT and neuropsychological measures.

D. UNIT ON BRAIN IMAGING AND COMPUTERS (BARRY HORWITZ, CHIEF)

This unit is responsible for conducting research involving in vivo structural imaging of the human brain in healthy subjects and in the patient groups noted above. Images are obtained using magnetic resonance imaging (MRI). Quantitative volumetric analyses are performed in order to assess differences in volumes of significant brain structures (e.g., ventricles, basal ganglia), and to determine volumetric changes in individuals followed longitudinally. This Unit also conducts research on human in vivo brain phosphorus and glucose metabolism using magnetic resonance spectroscopy (MRS). In addition, this Unit conducts research involving the use of multivariate statistical methods and computer computational techniques for analyzing brain functional activity as measured by PET.

E. SECTION OF NEUROCHEMISTRY AND BRAIN TRANSPORT (QUENTIN R. SMITH, CHIEF)

The function of this section is to conduct research on the transport, distribution, metabolism, and physiological actions of critical solutes within the central and peripheral nervous systems in relation to brain function, aging and dementia. The program examines the cerebral uptake, distribution and actions of environmental toxins and metals which may have a role in brain aging and dementia. In addition, the program explores the mechanisms that regulate cerebral metabolism, protect the brain from circulating toxins, and maintain a stable ionic environment for neuronal function.

F. UNIT ON NEUROPSYCHOLOGY (JAMES V. HAXBY, CHIEF)

This unit is responsible for the design, implementation and analysis of neuropsychological research on memory, language, cognition, and attention in healthy subjects and in patient groups noted above. The goal of this research is to identify and describe changes in mental abilities that are a function of age or age-related disease, to propose and test cognitive models for these changes, and to relate them to neuroanatomical, neurochemical, and physiological changes that are concurrently measured. The unit participates in clinical protocols to evaluate cognitive and behavioral effects of centrally acting drugs, including possible therapeutic agents for the treatment of Alzheimer's disease.

G. UNIT ON PHARMACOLOGY AND PHARMACOKINETICS (TIMOTHY T. SONCRANT, CHIEF)

This unit is responsible for conducting research on the sites and modes of action of centrally acting drugs in humans, in relation to peripheral pharmacokinetics, behavioral and cognitive responses and metabolic changes within the brain. Humans are studied in relation to age and neurodegenerative disorders, including Alzheimer's disease and depression. Drugs are evaluated for therapeutic efficacy, using cognitive and other measures. Cerebrospinal fluid concentrations of neurotransmitters and their metabolites are measured by analytical techniques, often developed by the unit. The unit also trains clinicians in the proper conduct of research in clinical pharmacology and therapeutics.

II. RESEARCH HIGHLIGHTS

This section summarizes selected research accomplishments from the Office of the Chief (Stanley I. Rapoport) and Section on Cerebral Physiology and Metabolism, not summarized under later Section or Unit Headings.

A. SECTION ON CEREBRAL PHYSIOLOGY AND METABOLISM

I. MECHANISMS FOR ALZHEIMER DISEASE

1. Evolution of the primate brain. It was proposed that expansion and differentiation of individual brain regions during primate evolution occurred in a systematic manner, consistent with functional neuroanatomy, and corresponded to the cointroduction of higher order cognitive functions and behaviors. The coherent evolution of certain groups of regions, organized as functional "systems", was described in terms of a new principle of "vertical" evolution termed "integrated phylogeny." A recently expanded system was shown to be selectively vulnerable to Alzheimer disease. This work was done by S. Rapoport.

2. Phylogenetic hypothesis for Alzheimer disease. Positron emission tomography demonstrates selective metabolic involvement of the frontal, parietal and temporal association neocortices in Alzheimer patients, and relative lack of involvement of primary and sensory motor regions. Furthermore, Alzheimer neurofibrillary tangles are selective to the association as compared to primary sensory and motor cortical regions, and Alzheimer neuropathology is found in nonneocortical brain regions which underwent rapid changes during recent hominid and higher primate evolution. These observations suggest that Alzheimer disease is a phylogenetic disease which involves a system of brain regions which underwent rapid expansion during evolution of higher primates. The genetic changes that promoted the evolution of these regions in higher primates may be related to their vulnerability to Alzheimer-type degeneration. This work was done by S. Rapoport.

3. Reversible synaptic failure underlies early functional deficits in Alzheimer disease. A hypothesis was put forward that early functional and metabolic deficits in Alzheimer disease reflect reversible failure of synaptic transmission. This hypothesis was based on evidence from our laboratory (see below) that brain association areas which demonstrate reduced resting glucose metabolism and blood flow can nevertheless be fully activated during an appropriate cognitive task; and on evidence that synapses are lost in excess of neurons early in Alzheimer disease. Reversible synaptic failure suggests that therapy be directed to prevent further loss of synaptic circuitry, and to optimize the circuitry which remains intact in early Alzheimer disease. This work was done by S. I. Rapoport and C. Grady.

4. Region-specific membrane instability in Alzheimer disease. The arrangement of cell membrane lipids within a stable bimolecular layer depends on membrane composition and body temperature. *In vitro* membrane lipids from the brain spontaneously assemble into a unilamellar state at a critical temperature T* of 37°C. T* is reduced by 10°C in affected but not unaffected regions of the brain in Alzheimer disease. Thus, cell membranes in these affected regions are physically unstable, and would tend to become disrupted over time. Their rate of disruption may influence the course of Alzheimer disease. This work was done by L. Ginsberg, N. Gershfeld and S. I. Rapoport.

II. MOLECULAR BIOLOGY OF BRAIN AGING AND DISEASE

1. Differential gene expression in the primate brain: relation to Alzheimer disease. A frontal pole cDNA library from the rhesus monkey brain was found to have cDNA clones which were expressed much more than in primary sensorimotor cortical regions. Three clones were mitochondrial DNA encoded genes for the cytochrome oxidase subunits I, II and III, reflecting a greater density of mitochondria and neuropil (synapses and dendrites) in association than in primary cortices in the primate brain. Such differences in gene expression may

be related to selective vulnerability of association cortical regions to Alzheimer disease in humans. This work was done by K. Chandrasekaran.

2. Cytochrome oxidase in the hippocampal formation of the primate brain. Cytochrome oxidase activity was identified by cytohistochemistry in terminal fields of long projection neurons in the monkey brain, within the perforant pathway of the hippocampal formation and entorhinal cortex, and within the neocortex. On the other hand, cytochrome oxidase mRNA was found within neurons leading to these projections using in situ hybridization. These results indicate that long projection neurons, which are particularly vulnerable to Alzheimer degeneration in humans, have the enzymatic machinery and synthetic capacity for high levels of oxidative metabolism. As such, they may be the sites of mitochondrial abnormalities or of accumulation of free radicals. This work was done by K. Chandrasekaran and D. Brady.

3. Cytochrome oxidase gene expression in the Alzheimer brain. Examination of postmortem Alzheimer brain using classical staining techniques and in situ hybridization for mRNA for cytochrome oxidase II, demonstrated more than 75% losses in cells of the perforant pathway of the hippocampal formation and entorhinal cortex, parts of the brain association system that is selectively vulnerable to Alzheimer disease and that has evolved dramatically in humans (see I.2, above). These results suggest that Alzheimer type degeneration is related to high levels of oxidative metabolism in certain synaptic areas, and perhaps to mitochondrial abnormalities, synaptic dysfunction or free radical accumulation (see I.3 above). This work was done by K. Chandrasekaran and D. Brady.

4. Parvalbumin in the Alzheimer brain. This calcium buffering protein was reduced significantly in the lateral and basolateral nuclei of the amygdaloid complex of the Alzheimer brain. These nuclei are part of the brain association system which is vulnerable to disease, as compared to other nuclei within the amygdala. These studies indicate again that systems connectivity and vulnerability must be considered in examining Alzheimer disease pathogenesis. This work was done by Dr. D. Brady.

5. Amyloid precursor protein (APP) synthesis and distribution. APP, thought to be pathogenic in Alzheimer disease, was shown to be selectively induced by nerve growth factor in differentiating PC12 and PC12S cells, and to localize in growth cones of neurites of PC12S but not PC12 cells. The form induced was APP695. These results indicate a selective interaction between nerve growth factor and APP695 expression. This work was done by R. Fukuyama.

6. Hippocampal transplants from the trisomy 16 mouse, a model for trisomy 21 (Down syndrome). Hippocampal neurons from embryonic day 15-17 trisomy 16 and control mouse fetuses were transplanted into brains of 6-8 week old adult mice, and allowed to survive for 6-14 months. No evident morphological, immunological or molecular difference was noted between control and trisomic transplants, each of which was robust even at 14 months. These results show that despite having genes which correspond to those on human chromosome 21, including the gene for amyloid precursor protein, excess expression of the 16th chromosome in the mouse does not lead to Alzheimer type degeneration in neural tissue. The results are consistent with the hypothesis that full blown degeneration needs the human genome in its entirety, and that Alzheimer disease may be phylogenic (See A.2 above).

III. REGULATION OF NEURONAL DEVELOPMENT

1. Trisomy 21 fetal dorsal root ganglion neurons in culture: effect of nerve growth factor. Dorsal root ganglion neurons from Down syndrome and normal human fetal abortus tissue were plated and cultured for 7-14 days with or without nerve growth factor (NGF). Survival rate was reduced from 98% to 37% by absenting NGF from the medium. With or without NGF, trisomic neurons showed a shortened action potential, and acceleration of both depolarization and repolarization as compared with control neurons. In the absence of NGF, rates of repolarization and depolarization were reduced in both neuron types. These

data show distinct differences in active electrical properties between Down syndrome and control neurons, and that NGF changes these properties in the same direction in both neuron types as the trisomy 21 defect. Trisomy 21 may affect a regulatory mechanism for the action potential which also can be regulated by NGF. This work was done by P. Caviedes.

2. Electrophysiological properties of hippocampal neurons from the trisomy 16 mouse, a model for Down syndrome in humans (trisomy 21). Use of patch clamp techniques on fetal mouse hippocampal neurons in culture showed that trisomy 16 neurons had a reduced rate of depolarization of the action potential as compared to control hippocampal neurons, and a reduced voltage-dependent sodium inward current. Those differences are in the opposite direction from observed in dorsal root ganglion human trisomy 21 and mouse trisomy 16 neurons, and these suggest neuron specific abnormalities rather than a general difference in active electrical properties in the trisamic state. This work was done By E. Coan and Z. Galdzicki.

3. Calcium currents in cultured trisomic hippocampal neurons. Whole cell inward calcium currents, recorded from the somatic region of cultured mouse hippocampal control and trisomy 16 neurons, were identified as high and low voltage activated currents. The high but not low voltage activated calcium current was significant larger in the trisomic neurons, indicating another difference in active electrical properties which might be related to retardation in Down syndrome. This work was done by Z. Galdziki and E. Coan.

IV. BRAIN LIPID METABOLISM, RELATION TO FUNCTION AND AGING

1. Mathematical model for incorporation of plasma fatty acids into brain phospholipids. A model was developed to quantitate incorporation of intravenously injected radiolabeled fatty acids into brain phospholipids in vivo. The model includes entry of the fatty acid from plasma into brain, oxidation of the fatty acid, *de novo* synthesis from acetate, and recycling from acyl-containing brain lipids. Unidirectional incorporation coefficients, calculated by an operational equation, can be used to estimate turnover of particular fatty acids in individual phospholipids. The model provides a basis for using the fatty acid method in humans, with positron emission tomography. This work was done by P. Robinson and S. I. Rapoport.

2. Incorporation of radiolabeled fatty acids into an experimental brain tumor. Rats with brain implants of Walker 256 carcinosarcoma cells were injected with radiolabeled palmitate, docosahexaenoate or arachidonate, and incorporation into and turnover within tumor and brain phospholipids, of each of the fatty acids, were measured using the fatty acid model of Robinson and Rapoport (Brain Res Revs, in press; IV.1, above). Marked labeling by up to 7 fold, by each of the fatty acid tracers, of tumor as compared with control brain tissue, was noted. This labeling corresponded to up to 10-fold increases in calculated upper bounds for turnover of the fatty acids within individual tumor phospholipids, including sphyngomyelin, as compared to normal brain. Rates of incorporation into tumor were in the order: palmitate > arachidonate > docosahexaenoate. These results suggest that the fatty acid method can be used to characterize the metabolism of and to image human brain tumors, provided appropriate tracers and imaging techniques can be developed (see below). This work was done by T. Nariai, J. DeGeorge and N. Greig.

3. Up regulation of post-synaptic cholinergic receptors following a chronic lesion of the nucleus basalis of Meynert in the rat, as measured with the fatty acid method. Two weeks after a unilateral lesion of the nucleus basalis, the major source of cholinergic input to the neocortex, awake rats were administered [1-14C]arachidonate to examine turnover of phosphatidylinositol and phosphatidylcholine sn-2 position fatty acids. Increased incorporation of tracer into the cortex ipsilateral to the lesion demonstrated upregulation of post-synaptic systems involving arachidonate. Such compensatory regulation may also occur in Alzheimer disease, where cells of the nucleus basalis are lost. This work was done by T. Nariai and Y. Lamour.

4. Radiosynthesis of [1-C-11]arachidonic acid. In order to examine fatty acid incorporation into the human brain, using positron emission tomography, [1-C-11]positron emitting isotopes of arachidonate, palmitate and docosahexaenoate were synthesized and purified to 95%, using a synthetic step to synthesize precursors of critical Grignard reagents. This work was done by M. A. Channing.

5. Brain uptake of a radiolabeled fatty acid, as measured with positron emission tomography. The positron emitting fatty acid, [1-C-11]arachidonate, was injected intravenously in anesthetized rhesus monkeys and shown to produce quantifiable images of the brain; the calculated rate of incorporation into brain was similar to that found in the rat using [1-14C]arachidonate and autoradiography. Tracer uptake was independent of cerebral blood flow. The results indicate that positron emitting fatty acids can be used to examine brain phospholipid metabolism in higher primates and possibly in humans, with positron emission tomography. This work was done by R. Carson, W. Wakabayashi and L. Freed.

V. BLOOD-BRAIN BARRIER AND CENTRAL NERVOUS SYSTEM FUNCTION

1. Chemical modification of water soluble drugs for treating brain tumors. The alkylating anticancer agent, chlorambucil, which normally enters the brain very slowly and is ineffective against brain tumors, was converted into a tertiary butyl ester. This product was found to have optimal brain uptake and a long plasma half-life (due to steric hindrance of plasma esterases), while retaining significant alkylating activity. It also was active against human brain tumors *in vitro*, which were insensitive to chlorambucil, and is entering clinical testing. Thus, water-soluble drugs can be appropriately modified to increase brain uptake and half-life in plasma, for treating central nervous system disease. This work was done by N. Greig.

2. Development of anticholinergics for treatment of Alzheimer's disease. The loss of cortically-projecting basal forebrain cholinergic neurons characterizes the brain in Alzheimer disease and may contribute to memory deficits in that disorder. Physostigmine, an anticholinesterase, has been used to treat Alzheimer disease with limited success, possibly because of its short plasma half-life and lack of specificity for brain acetylcholinesterase. A large number of physostigmine derivatives were synthesized, with substitutions in the carbamoyl and N(1) positions, and were shown to have prolonged plasma half-lives and anticholinergic actions, and increased specificity for acetyl- as compared to butrylcholinesterase. These agents are being considered for further testing. This work was done by N. Greig and colleagues.

3. Osmotic modification of blood-brain barrier for brain tumor chemotherapy. Inadequate delivery of water-soluble drugs to the brain frequently is due to an intact blood-brain barrier, and accounts for many poor therapeutic responses of patients with brain tumors. Delivery can be enhanced in humans or animals by osmotic opening of the blood-brain barrier, following an intracarotid infusion of a concentrated sugar (arabinose, mannitol) solution. The procedure is being considered for controlled Phase III clinical trials for treating patients with primary cerebral lymphoma, in whom Phase II clinical trials show significant prolongation of life. This work was done by S. I. Rapoport and colleagues.

4. Development of therapeutics for treatment of drug abuse. Cocaine, a major drug of abuse, is thought to act by inhibiting reuptake of biogenic amines; its ability to inhibit dopamine uptake is thought to cause its addictive action. To see whether inhibitors of dopamine reuptake could act as a cocaine antagonist, the high affinity inhibitor GBR 12909 was studied and was shown to effectively inhibit the ability of cocaine to elevate extracellular brain dopamine. This work was done by N. Greig and R. Rothman.

5. Therapeutics for treatment of brain sequestered human immunodeficiency disease. The brain is an early site of HIV infection, and encephalopathy frequently becomes apparent. A variety of dideoxynucleoside analogs have been developed to inhibit reverse transcriptase of HIV in the brain, including AZT and DDC; these analogs can be metabolized in mammalian cells and inhibit HIV replication at doses that are not antiproliferative. Lipophilic derivatives of DDC were developed to increase and maintain high therapeutic levels of drug in brain. This work was done by N. Greig and P. Torrence.

B. SECTION ON BRAIN AGING AND DEMENTIA

I. GENETICS OF ALZHEIMER'S DISEASE

1. Transmissibility of Alzheimer disease. Blood was obtained from nine unaffected family members from two large pedigrees with neuropathologically confirmed autosomal dominant Alzheimer disease, six demented patients with clinically diagnosed Alzheimer disease, and two healthy controls without history of familial neurological disease, to examine a report that a spongiform encephalopathy similar to Creutzfeld-Jacob Disease can been transmitted to hamsters by intracerebral inoculation with buffy coat from blood of patients with Alzheimer disease and their unaffected first-degree relatives. After more than 500 days since the beginning of the second passage, no hamster in either the first or second passage developed neurologic signs. Blinded neuropathologic examination of sections of all first passage and most second passage hamster brains revealed no evidence of a spongiform encephalopathy. This work suggests that Alzheimer disease is not due to a transmissible agent. This work was done by M. Schapiro and M. Godec.

2. Dementia without mental retardation in partial mosaic trisomy 21. A 45-year-old woman with a mosaic partial translocation (duplication of g arm of chromosome 21) was shown to be demented but did not have a history of mental retardation (she was a bank clerk). Metabolic and CT studies confirmed Alzheimer type changes. This study suggests that dementia and mental retardation are caused by abnormalities at separate loci on chromosome 21, and that mental retardation is related with regard to chromosome 21. This work was done by M. Schapiro.

3. DNA Repair. PHA-stimulated blood lymphocytes from Down syndrome subjects showed increased chromatid aberrations, and DNA strand breaks and gaps in response to G2-phase-irradiation compared to Alzheimer's disease and normal cells. An abnormal response to post-irradiation addition of cytosine arabinoside, a DNA repair inhibitor, also was shown in Down syndrome cells, indicating a deficiency in incision at sites of DNA base damage. Thus, Down syndrome, like other cancer-prone genetic disorders, has a G2 DNA repair deficiency not associated with neurodegeneration. This work was done by M. Schapiro and K. Sanford.

4. Discordance and concordance in monozygotic twins indicates two modes of transmission of Alzheimer disease. Monozygotic twins concordant for dementia of the Alzheimer type or proven Alzheimer disease were shown to have a significantly higher frequency of a positive family history in at least one first-degree relative ($p<0.001$) than discordant monozygotic twins; and a lesser predicted distribution of proportions of surviving first degree relatives without disease ($p<0.001$). These observations suggest that concordant but not discordant monozygotic twins have a heritable form of disease, and that both heritable and nonheritable forms of Alzheimer disease exist. Alzheimer disease in discordant twins may result from environmental influences or a somatic chromosomal change following a zygotic division. This work was done by S. Rapoport, K. Pettigrew, and M. Schapiro.

II. BRAIN FUNCTION IN AGING AND DEMENTIA

1. Reductions in parietal/temporal glucose metabolism are not specific for Alzheimer's disease. A 68 year old man with slowly progressive dementia was diagnosed as having Alzheimer disease during life, but was found to have Parkinson's disease at autopsy. Reductions in relative metabolism as measured by positron emission tomography in parietal and temporal areas were shown, similar in regional distribution and magnitude to those seen in patients with probable Alzheimer disease. These results suggest that reductions of glucose metabolism in association neocortex in Alzheimer's disease are not specific to the disease process, but maybe related to the dementia state. This work was done by M. Schapiro.

2. Resting brain glucose metabolism does not identify subjects "at risk"for familial Alzheimer disease. A 65 year old man with memory complaints and family history of autosomal dominant Alzheimer's disease showed no consistent hippocampal, amygdaloid, or neocortical glucose abnormalities. This suggests that positron emission tomography is not useful for the diagnosis of Alzheimer disease in "at risk" subjects. Sequential resting PET studies, with MRI-PET superimposition for limbic regions, or stimulation paradigms may be required to diagnose "at risk" subjects. This work was done by M. Schapiro and P. Pietrini.

III. CEREBROSPINAL FLUID CHEMISTRY IN AGING AND DEMENTIA

1. Lumbar cerebrospinal fluid choline in Down syndrome. Choline in cerebrospinal fluid is in part a product of breakdown of acetylcholine within the central nervous system. Young adults with Down syndrome (a model for Alzheimer disease) were found to have elevated cerebrospinal fluid levels of choline, but not of the peptides somatostatin and neuropeptide Y, as compared to healthy age-matched controls, suggesting increased central cholinergic activity in Down syndrome. Such prolonged increased activity may contribute to the early appearance of Alzheimer-type degeneration is this disorder. This work was done by M. Schapiro.

C. UNIT ON POSITRON EMISSION TOMOGRAPHY

1. Cognitive activation of regional cerebral blood flow (rCBF) in aging. Eleven young (mean age 27 $\pm$ 4 yrs) and 9 old (mean age 72 $\pm$ 7 yrs) subjects were studied using positron emission tomography (PET). Both young and old subjects showed activation of superior parietal regions during a spatial task and of occipitotemporal regions during a object identification task. There was no difference in the magnitude of activation in these areas in the old subjects compared to the young subjects. However, the old subjects had more activation of superior parietal cortex during face matching and more activation of occipitotemporal cortex during dot-location matching, compared to the young subjects. These results suggest that dissociation of object and spatial vision in extrastriate cortex is maintained in the elderly, but that the two systems are less functionally distinct. This work was done by C. Grady, J. Haxby, and B. Horwitz.

2. Age-related changes in rCBF and rCMRglc during a global sensory activation. We studied young and old healthy men using PET with [15-O]-water and [18F]FDG under two experimental conditions- at rest and during sensory activation (watching a documentary film). Significant increases of rCBF and rCMRglc were found during sensory activation bilaterally in the occipital regions in both age groups. At rest, frontal rCBF and rCMRglc were higher in the young than in the old; sensory activation induced frontal increases in the young and decreases in the old. Coupling between rCBF and rCMRglc was present in both age groups and was stronger during sensory activation. These results suggest that rCBF and rCMRglc undergo more age-related changes in frontal than in other brain regions, and support brain activation as an ideal condition to enhance age-related difference in brain metabolism. This work was done by B. Horwitz and P Pietrini.

3. Activation of rCBF in patients with dementia of the Alzheimer type (DAT). Mildly-moderately demented DAT patients showed rCBF activation in occipitotemporal cortex in the same location as did the controls. In addition, the magnitude of the activation was not reduced compared to control levels (17% vs. 15% normalized increase). This was in spite of the fact that some patients had reduced flow in this region of cortex. The DAT patients also had increased rCBF during face matching in right frontal cortex, and area not activated in controls. These results indicate that extrastriate cortex retains the ability to locally increase CBF during task performance despite reductions in flow due to disease, and that additional areas of frontal cortex are needed, possibly due to an increased demand on attention in the patients. This was done by C. Grady, J. Haxby, B. Horwitz, and M. Schapiro.

4. Glucose metabolism and quantitative neuropathology in Alzheimer disease. Neurofibrillary tangles showed a striking predilection for association cortical regions, whereas plaques and neuronal densities had a more even distribution in brains of Alzheimer disease patients. In addition, tangle densities were positively correlated with regional metabolic measures in 5 of the 6 patients, whereas plaque density and neuronal counts were not related to metabolism. This study suggests that regional metabolic abnormalities seen during life are related to the pathological processes responsible for neurofibrillary tangle formation. This work was done by C. DeCarli, C. Grady, and M. Schapiro.

5. Longitudinal change in resting cerebral glucose utilization and clinical decline in patients with dementia of the Alzheimer type (DAT). Decreases in a ratio of association to primary cortex glucose metabolism were significantly correlated ($p<0.01$) with declines in overall cognitive measures. Measures of right/left metabolic asymmetry and neuropsychological discrepancy did not change significantly over the follow-up period; however, directions of both metabolic and neuropsychologic asymmetries remained stable over time. These findings demonstrate that overall cognitive changes in DAT reflect underlying changes in association neocortex as measured by PET. This work was done by J. Salerno, J. Haxby, and C. Grady.

6. Analysis of rCMRglc in subjects "at risk" for probable Alzheimer Disease. Two PET examinations one year apart were performed on a 65 y.o. man with isolated memory impairment and family history for autosomal dominant AD. Sixteen sex- and age-matched healthy volunteers were used for comparison. Initial rCMRglc data did not reveal any consistent abnormality as compared to controls, but a follow-up evaluation did reveal rCMRglc reductions in parietal regions, coincident with worsening of cognitive impairment. Using a discriminant function that successfully distinguished mild/moderate DAT patients from controls, the subject was correctly identified as an AD patient with 100% probability on both PET scans. These results emphasize the importance of alternative methods of analysis to increase usefulness of resting PET data in the early detection of AD. This work was carried out by P. Pietrini and N. Azari.

7. DAT with Visual Symptoms. Two patients with prominent visual symptoms (VS) were compared to 16 DAT patients without visual symptoms to see if metabolic defects in visual areas accompanied these symptoms. Normalized rCMRglc values were found to be significantly lower in the VS patients in primary occipital and parietal and occipital association cortices bilaterally. These metabolic results are consistent with pathological reports of a subgroup of AD patients who show visual symptoms and increased degenerative changes in striate and other occipital cortex compared to the majority of AD brains. This work was conducted by E. Micheli and P. Pietrini.

8. Parkinson's disease vs. Alzheimer Disease. A patient was found to have Parkinson's disease at autopsy. A PET scan performed prior to death showed reduced glucose metabolism in parietal and temporal cortex, similar in location and magnitude to that seen in DAT patients. This suggests that reductions of

glucose metabolism in association neocortex may not be specific the disease process, but may be related to the dementia state. This work was done by M. Schapiro, C. Grady, and C. DeCarli.

9. Hypertension. All hypertensives were free of apparent end-organ impairment and were cognitive normal. Using a Z score analysis 29% of hypertensives and 4% of controls had hypometabolism in 4 or more brain regions. Hypertensives also had significantly lower metabolism in 13 regions that were clustered in the vascular territory of the perforating arteries or in the middle/anterior cerebral artery watershed areas. This suggests that long-standing treated hypertension can lead to subclinical brain dysfunction, and that watershed and subcortical structures are at greatest risk. This work was done by J. Salerno, M. Schapiro and C. DeCarli.

10. Cerebrovascular Dementia. Ten patients with dementia and deep white matter changes on MRI (DWMC) were compared to 23 age and severity matched DAT patients and 46 healthy controls. Patients with DWMC had significantly increased prevalence of hypertension, focal neurologic signs and infarcts on CT or MRI. Mean global glucose metabolism as measured by PET was not different between the two demented groups. Ratios of regional to global metabolism were significantly lower in subcortical regions and higher in parietal regions in DWMC patients compared to those in DAT patients. We conclude that patients with DWMC have increased prevalence of cerebrovascular disease and show a different pattern of cerebral metabolic deficit than do patients with DAT. This work was done by C. DeCarli and C. Grady.

11. Trichotillomania. We examined glucose metabolism in trichotillomania (TM), a disease similar to Obsessive-Compulsive Disorder (OCD). Global metabolism was significantly increased in the group of 10 adult females with TM when compared with a group of 20 normal female controls, but none of the areas postulated to be involved in TM, based on our hypothesis that TM might be part of a spectrum of obsessive-compulsive behaviors, showed differences between TM patients and controls. However we did find a significant correlation between response to clomipramine and metabolism in the orbitofrontal and anterior cingulate regions, as was found in the OCD patients. This work was conducted by C. Grady, M. Schapiro, and S. Swedo.

D. UNIT ON BRAIN IMAGING AND COMPUTERS

1. Quantitative CT analyses in healthy aging in men and women. The relations between age and gender and ventricular volumes were determined for 64 healthy men and 43 healthy women. Gender differences in ventricular volume were found (women having smaller left and right lateral ventricles than men), as was increased ventricular size with advancing age. Decline in cognitive processes (measured by the WAIS) and increased ventricular volume seem to be relatively independent processes correlated more to the age of the subject. This work was conducted by J. Kaye and C. DeCarli.

2. Longitudinal CT changes in dementia of the Alzheimer type (DAT). Rates of lateral ventricular enlargement and decline in cognitive performance for 11 men and nine women with Dementia of the Alzheimer type (DAT) were determined and compared to the same measures obtained for nine male and eight female age-matched healthy controls. The rate of total lateral ventricle enlargement (cm^3/year) was significantly different between DAT and healthy controls, and was more specific and sensitive to the diagnosis of DAT than comparison of cross-sectional volumes at final evaluation. The rate of total lateral ventricular enlargement very early in the course of Alzheimer's disease was significantly less than the rate of ventricular enlargement after the onset of nonmemory cognitive deficits, but stable for all other degrees of dementia severity, which suggests a biphasic process. This work was performed by C. DeCarli and J. Haxby.

3. Magnetic resonance imaging and temporal lobe volumes in healthy aging. Temporal and posterior frontal brain volumes were quantified from coronal MRI scans in a cross-sectional study of 30 very healthy men aged 21-92 years. A

significant age-related decrease (approximately 1% per decade) of posterior frontal, but not of temporal lobe volume was found. This research was performed by C. DeCarli and B. Horwitz.

4. CSF and subcortical nuclei in healthy aging. Healthy male subjects free of primary brain disease were studied using MRI to determine age differences in the volumes of gray matter nuclei, cerebral ventricles, cerebral brain matter volume and cerebrospinal fluid (CSF). Significant age related atrophy in the lenticular and caudate nuclei was found, as was increased CSF in the elderly subjects. The aging process was found to affect the caudate and lenticular nuclei significantly more than the cerebral hemispheres. This work was performed by D. Murphy and C. DeCarli.

5. MRI volumetric changes in men with DAT. A quantitative morphometric MRI study of the brain in DAT was performed to see if cognitive decline in DAT patients was related to brain morphometric changes. DAT men had significantly less cerebral brain matter and significantly larger volumes in every CSF measure (except right and left peripheral CSF) than old healthy men. Men with mild DAT had significantly less cerebral brain matter and significantly larger lateral ventricles than old healthy men. Neuropsychological measures of disease severity in DAT patients were significantly and appropriately correlated to the normalized volumes of cerebral brain matter and right lateral ventricle. This work was performed by D. Murphy, C. DeCarli and B. Horwitz.

6. Ventricular changes in controlled hypertension. Neurologically and cognitively normal hypertensives were studied with volumetric MRI to examine whether hypertension influenced age-related structural changes in the brain. Mean volumes of both the right and left lateral ventricles were significantly larger in the hypertensives as compared to controls. These results suggest that structural changes may represent pre-clinical alterations in the brain due to longstanding hypertension. This work was performed by J. Salerno.

7. Magnetic resonance spectroscopy. PET, MRI and magnetic resonance spectroscopy (MRS) were used to study brain glucose (rCMRglc) and ^{31}P phosphorus metabolism in DAT patients and matched control subjects. PET, MRI, and MRS were all carried out on the same volume of brain matter, from 3.5 - 6.5 cm above the inferior orbitomeatal line. Mean rCMRglc was significantly lower in the DAT group than controls. However, no significant group difference in any phosphorus metabolite concentration/ratio was found, and no significant correlation between any phosphorus metabolite concentration/ratio and severity of dementia, or glucose metabolism was obtained, suggesting that glucose metabolism is reduced early in DAT (reflecting decreased basal synaptic functioning), and is unrelated to a rate-limitation in glucose delivery, abnormal glucose metabolism, or abnormal coupling between oxidation and phosphorylation. Normal or near normal levels of phosphorus metabolites are maintained at all stages of DAT. This work was done by D. Murphy, B. Horwitz, J. Alger, P. Bottomley, C. DeCarli and J. Salerno.

8. Brain Metabolic Patterns in Obsessive-Compulsive Syndrome. Correlation analysis of rCMRglc PET values was used to investigate functional associations between pairs of brain regions in 18 adult patients with primary obsessive-compulsive disorder (OCD) of childhood-onset, as compared with 18 age- and sex-matched control subjects. Correlations involving the caudate nuclei did not differ between the two groups for the most part. The anterior medial temporal region (which includes the amygdala) had correlations in the OCD group that were significantly larger with frontal areas than in controls, and correlations that were significantly smaller with posterior brain regions. These results suggest that the correlation pattern in OCD is not characterized by an overall loss of functional integration, but rather, by functional reorganization. Moreover, because brain structures in the AMT area appear to play a crucial role in integrating environmental cues with input from the internal milieu, the abnormal pattern of correlations involving the AMT in OCD may indicate that OCD subjects have difficulty relating externally and internally generated neural information. This work was performed by B. Horwitz and S. Swedo.

9. Gender Differences in Correlations of Cerebral Glucose Metabolic Rates in Young Normal Adults. The correlation analysis of rCMRglc PET values was applied to resting FDG data collected from 15 women (age 21-38 yr) and 18 men (age 21-38 yr). Although the total number of significant correlations did not differ between men and women, patterns of correlations differed: correlations for women r(F) were more positive than correlations for men r(M) more often than r(M) > r(F). Most of the r(F) > r(M) cases involved left-hemisphere frontal (26%) ROIs; most of the r(M) > r(F) cases involved right-hemisphere sensorimotor (28%) and occipital (25%) ROIs. The findings demonstrate gender differences in patterns of neocortical interactions at the 'resting' state which may reflect gender differences on neuropsychological and neuroanatomical measures. This work was done by N. Azari and B. Horwitz.

10. Age Differences in Correlations of Cerebral Glucose Metabolic Rates in Normal Adults. The correlation analysis was applied to resting FDG data collected from 15 young (age 21-38 yr) and 17 old (age 65-90 yr) women using a high resolution Scanditronix tomograph. Old women had smaller frontoparietal correlations than young women. This result corroborates our previous correlational analysis applied to data collected with the lower resolution ECAT II tomograph. This work was performed by N. Azari and B. Horwitz.

11. Differences in Patterns of Cerebral Glucose Metabolic Rates in Alzheimer's Patients. Correlation analysis was applied to resting normalized rCMRglc data collected using the Scanditronix PC1024-7B tomograph from 19 patients (12 men, 7 women) with mild-to-moderate dementia of the Alzheimer type (DAT) (ages: 52-81 yr) and 22 healthy, age-matched controls (ages: 53-75 yr). The DAT group had fewer positive significant correlations in the whole brain than controls. Values in the DAT group generally were smaller than in controls for frontoparietal, frontal-sensorimotor, frontal-limbic and sensorimotor-parietal correlations. The results corroborate our previous work using a low resolution scanner showing decreased frontoparietal associations in mild/moderate DAT patients in the 'resting' state, and demonstrate decreased interactions between regions which modulate attention. This research was done by N. Azari and B. Horwitz.

12. Comparison of cerebral metabolic patterns in mild hypertensive patients vs. controls. A correlation analysis was applied to resting normalized rCMRglc data from 17 mildly hypertensive men without cognitive deficits, who have been on antihypertensive therapy for more than 10 years, and 25 age-matched healthy men. A region of interest template was created which allowed correlations between vascular areas to be performed. Analysis of all the brain's significant correlation differences (scd), showed that for the brain as a whole, there were significantly more scd's in which the hypertensive group correlation value was the smaller of the pair. The vascular area most affected was the middle cerebral - anterior cerebral watershed area. This work was performed by M. Mentis, B. Horwitz and J. Salerno.

13. Functional coupling during visual processing. Correlational analysis was performed on normalized rCBF values, obtained by PET with [15-O]-labeled water, to examine functional interactions among brain regions in posterior neocortex in young men during two 2-choice, match-to-sample visual tasks: face discrimination (FD), and dot localization with rotation (DL). Although both tasks activated lateral occipital and occipitotemporal cortex bilaterally, correlational analysis revealed that during FD, occipital and occipitotemporal activations correlated significantly, but only in the right hemisphere. During DL, occipital and superior parietal activations correlated significantly, but again, only in the right hemisphere. These results support the view that FD and DL processing are carried out to a greater degree by the right posterior hemisphere. This work was performed by B. Horwitz, J. Haxby, C. Grady, and M. Schapiro.

14. Functional interactions in corpus callosotomized rat brain. Correlation coefficients among normalized rCMRglc for 97 brain regions and their contralateral homologues were examined in rats that had undergone corpus

callosotomy and in sham-operated controls. Correlation coefficients between left-right region pairs were more often lower than higher in callosotomized animals, indicating that the corpus callosum (CC) is important for functional interactions of these structures. This work was performed by T. Soncrant, S. Sato and B. Horwitz.

15. Functional interactions in rat brain following nucleus basalis magnocellularis ablation (nbm). Unilateral destruction of the nbm in rats reduces cholinergic inputs to the ipsilateral frontoparietal neocortex and disrupts behavior. Correlational analysis of rCMRglc was performed on 2 groups of 16 young rats at 2 wk after ablation of the right nbm with ibotenate or sham surgery. Most correlations between cholinergic nuclei (medial septum and diagonal band) and right but not left frontoparietal cortical regions were larger in lesioned rats, as were those between most frontoparietal region pairs. This work was performed by T. Soncrant, Y. Lamour and B. Horwitz.

16. Early detection of Alzheimer's disease (AD) using PET and discriminant analysis. A multiple regression/discriminant analysis procedure was applied to resting rCMRglc PET data in mildly/moderately demented AD probable patients and age-matched controls. The results demonstrated that a discriminant function, reflecting functional interactions involving frontal and parietal regions, successfully distinguished AD patients from controls, and identified an AD-like pattern in the first PET scan of the at risk subject (at which time the subject only showed mild memory impairment, was diagnosed with possible AD, and had a normal scan as assessed by traditional analytic methods; at the second evaluation, however, this subject was diagnosed with probable AD, and his scan showed typical AD hypometabolism). This work was performed by N. Azari, B. Horwitz, K. Pettigrew and J. Haxby.

17. Abnormal metabolic pattern involving language areas in Down syndrome (DS). The correlational method and the multiregression/discriminant analysis procedure were applied to resting rCMRglc PET data in young adults with DS to test the hypothesis of an abnormal pattern of rCMRglc involving language areas. A discriminant function, reflecting regional interactions involving the two primary language regions, successfully classified DS (100%) and control (88%) subjects. This work was performed by N. Azari, B. Horwitz, K. Pettigrew, J. Haxby and M. Schapiro.

18. Computer simulation model for correlational analysis of regional cerebral blood flow (rCBF) values. A computer simulation model was devised in order to provide a partial validation for correlation analysis as applied to rCBF data. Because the underlying pattern of functional couplings in the model is specified, these simulations demonstrate that the change in the correlation coefficient between normalized rCBF values reflects the change in the corresponding functional coupling. An example of the use of the simulation method showed how rCBF activations during a task (relative to a control state) could be bilateral in the brain, even though non-zero within-hemisphere functional couplings were found only in one hemisphere. This work was carried out by B. Horwitz.

E. SECTION ON NEUROCHEMISTRY AND BRAIN TRANSPORT

I. BRAIN NUTRIENTS AND METALS IN AGING AND DISEASE

1. Identification of the blood-brain barrier transporter for basic amino acids. The bloodbrain barrier controls the brain access of many critical nutrients through the selective expression of various carrier- and receptor-mediated transport systems. As a first step towards isolating and cloning these carriers, the mRNA of rat brain capillaries was examined by Northern blot analysis and shown to express high levels of mRNA for the sodium-independent basic amino acid carrier. Transporter mRNA levels in brain capillaries exceeded that in surrounding tissue by >10 fold. This work was done by J. Stoll, K. Wadhwani and Q. Smith.

2. Brain delivery of hydrophilic drugs via carrier-mediated transport across the blood-brain barrier. Rat brain perfusion studies identified six hydrophilic anticancer drugs that are transported into brain via the large neutral amino acid carrier of brain capillaries. One agent, D,L-NAM, exhibited markedly high affinity for the carrier (K_m <0.2 uM) and was taken up into brain 20-40 times more rapidly than its clinical analogue, L-melphalan. The results demonstrate the feasibility of improving drug delivery to brain through the design and synthesis of high affinity analogs to the cerebrovascular nutrient carriers. This work was done by Q. Smith and Y. Takada.

3. Saturable transport of manganese(II) at the blood-brain barrier. Manganese(II) influx from plasma into brain was examined in rats and found to be saturable with a V_{max} of 0.018 nmoVmin/g and a K_m of 1 uM. A nonsaturable component was also present in some brain regions. The results demonstrate that the blood-brain barrier has a saturable transporter that regulates the brain uptake of manganese, an essential metal ion. This same transporter may facilitate the brain uptake of toxic metals, such as lead, cadmium and aluminum. This work was done by V. Murphy and K. Wadhwani.

4. Influence of dietary calcium deficiency on brain metal uptake and devosition. Young rats were fed diets either low or normal in calcium, with and without supplemental aluminum, lead, cadmium or manganese, for 6 to 8 weeks. At the end of the exposure period, brain concentrations for each of the four metals increased significantly in animals fed low calcium diet as compared to control animals. Low calcium intake also produced a precipitous drop (>90%) in serum copper concentration, a lessor decline in brain copper (~20%), and significant imbalances in brain concentrations of a number of other essential ions. The results demonstrate the critical influence of calcium deficiency and diet on brain metal uptake and toxicity. This work was performed by V. Murphy and J. Rosenberg.

5. Brain uptake of beta-N-methylamino-L-alanine. BMAA, the putative neurotoxin of the high incidence amyotrophic lateral sclerosis-parkinsonism dementia complex of Guam, was administered to rats and found to be rapidly cleared from plasma in an initial distribution phase, followed by a slower elimination phase ($t_{1/2}$~1 day). Brain uptake was saturable and mediated by the cerebrovascular large neutral amino acid carrier. After two weeks of continuous infusion (100 mg/kg/day), brain concentrations equalled 10 - 30 ug/g. The results suggest that BMAA is taken up into brain and can reach potentially toxic levels following high-dose administration (> 100 mg/kg). This work was done by Q. Smith.

II. FUNCTIONS AND STRUCTURE OF PERIPHERAL NERVE

1. Blood-nerve barrier permeability and nerve vascular volume during aging. The blood-nerve barrier permeability to [14C]sucrose was examined in Fischer-344 rats using an intravenous injection technique and found to remain stable between 3 mo and 30 mo of life. Morphometric measurements of nerve vascular surface area and blood volume also did not change. The results suggest that the integrity of the BNB is maintained with age in the rat. This work was conducted by K. Wadhwani.

2. Saturable transport of amino acids and metals across the blood-nerve barrier. Nerve, like brain, requires certain metals and nutrients for normal metabolism and function. Many of these solutes, because of their poor lipid solubility, only poorly cross barrier membranes by passive diffusion. K. Wadhwani demonstrated that both essential neutral and basic amino acids, as well as manganese, are transported into nerve by saturable carriers at the blood-nerve barrier. The transporters, for the most part, had properties similar to those at the blood-brain barrier and other tissues. The results demonstrate that while the bloodnerve barrier differs in many respects from the blood-brain barrier, critical functions, such as facilitated uptake of essential nutrients and metals, are maintained.

3. Blood-nerve barrier integrity durin Wallerian de~eneration. The effect of degeneration of nerve fibers on the integrity of the blood-nerve barrier was examined in the frog following transection of the sciatic nerve. Permeabilities of both the nerve capillaries and perineurium increased during the first two weeks of Wallerian degeneration. During the next nine months the permeability of nerve capillaries returned to no~nal, whereas the permeability of the perineurium remained elevated. The results suggest that nerve cells are required to maintain the integrity of the perineurium, but not nerve capillaries. This work was conducted by C. Latker and K. Wadhwani.

F. UNIT ON NEUROPSYCHOLOGY

1. Activation and localization of cortical visual pathways. Studies of regional cerebral blood flow, as measured with ^{15}O and positron emission tomography (PET), during the performance of spatial and object vision tasks in healthy men, demonstrated two anatomically distinct and functionally specialized pathways, the locations of which were not absolutely predicted from studies in nonhuman primates. Young and old adults had rCBF increases with similar magnitudes and spatial distributions. Patients with mild Alzheimer's disease had rCBF increases during the object vision task that had the same neuroanatomical location and magnitude as that seen in controls, even though this regions demonstrated reduced rCBF. Patients with Alzheimer's disease additionally demonstrated activation in prefrontal regions not activated in healthy controls. Preserved capacity to increase rCBF suggests that the neural systems associated with recruiting association areas for cognitive processes are not impaired when the areas themselves are. The additional activation of frontal areas suggests a compensatory mechanism, reflecting the increased attentional load associated with face matching performance in patients with Alzheimer's disease. This work was done by J.V. Haxby, C.L. Grady, B. Horwitz, M.B. Schapiro, M. Mishkin, L Ungerleider, P. Herscovitch, and R. Carson.

2. Longitudinal course of neuropsychological impairment in DAT. Longitudinal study of patients with DAT for as long as five years has shown that there can be an initial plateau phase during which only memory is impaired, but decline on other neuropsychological functions is not observed. Afterwards, neuropsychological decline on nonmemory cognitive abilities is remarkably linear. The rate of decline varies markedly among patients, but the rate for an individual is quite constant. Consequently, future rate of decline can be predicted, based on longitudinal neuropsychological evaluations. After memory impairment, the first neuropsychological functions to demonstrate significant deficits in patients with mild DAT are attention, planning and foresight, and abstract reasoning. Patterns of nonmemory neuropsychological impairments tend to persist over time, despite worsening severity of overall dementia. For example, patients with disproportionate verbal impairment relative to visuospatial impairment tend to demonstrate the same neuropsychological discrepancy at follow-up. This work was done by J.V. Haxby and C.L. Grady.

3. Metabolic and neuropsychological patterns are correlated in DAT. In patients with moderate Alzheimer's disease, the relative disproportion of language vis-a-vis visuospatial impairments was significantly correlated with right-left asymmetry of regional cerebral metabolic rates for glucose (rCMRglc). Moreover, discrepancies between calculations or immediate visuospatial memory span, on the one hand, and attention or verbal fluency, on the other, correlated significantly with metabolic discrepancies between parietal and frontal association cortices. In mildly demented patients, metabolic patterns were not correlated with nonmemory neuropsychological patterns. These patients had no significant impairments of nonmemory language and visuospatial functions with significant abnormalities of neocortical metabolism. Follow-up studies demonstrated significant deterioration of nonmemory neuropsychological functions, which correlated with right-left metabolic asymmetries. These findings confirm that neocortical metabolic changes precede the development of demonstrable neocortically mediated neuropsychological impairments in early DAT. It appears

that the brain is capable of compensating for these early physiological changes to maintain premorbid neuropsychological function. This work was done by J.V. Haxby and C.L. Grady.

4. Selective attention in healthy aging and DAT Cognitive studies of selective and shifting attention to visual features in compound stimuli identified two dissociable underlying processes, one driven by intention and the other requiring priming with practice. Patients with mild dementia of the Alzheimer type were found to have disproportionate impairments on tests of selective and divided attention to visual features. In a separate study, patients with DAT were found to have impaired selective attention to spatial location, such that they were less efficient at responding to stimuli in unattended locations. This impairment was correlated with superior parietal lobule rCMRglc, providing evidence for the neuroanatomical basis for this aspect of the attentional impairment in DAT. A study of selective and divided attention to sensory modality (visual and auditory) found that aging was associated with no change in the ability to attend selectively to one channel and increasing difficulty shifting attention from one sensory channel to the other. By contrast, patients with DAT were found to have impairment of both selective and shifting attention, although the impairment of shifting attention was more marked. This work was done by J.V. Haxby and A. Berardi in collaboration with R. Parasuraman and P. Greenwood of Catholic University.

5. Neuropsychological evaluation of adults with Down syndrome. Down syndrome subjects over 35 years of age, without clinical dementia, demonstrated significant neuropsychological abnormalities relative to young Down adults. Abilities to form new long-term memories and visuospatial construction were consistently diminished, whereas immediate memory span and language were not. These neuropsychological deficits may be the early consequences of Alzheimer's disease neuropathology. Longitudinal study of older adults with Down syndrome showed that onset of dementia is associated with accelerated decline of neuropsychological function, increased rate of cerebral atrophy, and the appearance of significant reductions of association cortex metabolism. These qualitative changes in brain function and anatomy suggest that dementia onset represents more than an arbitrary point in a continuous process of degeneration. Rather, dementia onset appears to signal a fundamental change in the AD process. A mental status examination for adults with Down syndrome was developed that provides good discrimination between adults with and without clinically significant dementia. This work was done by J.V. Haxby and M.B. Schapiro.

6. Neocortical metabolic abnormalities precede nonmemory cognitive impairment in DAT. DAT patients with mild impairment had no significant impairments of nonmemory language and visuospatial functions with significant abnormalities of neocortical metabolism Follow-up studies demonstrated significant deterioration of nonmemory neuropsychological functions, which correlated with right-left metabolic asymmetries. These findings confirm that neocortical metabolic changes precede the development of demonstrable neocortically-mediated neuropsychological impairments in early DAT. It appears that the brain is capable of compensating for these early physiological changes to maintain premorbid neuropsychological function. This work was directed by J.V. Haxby and C.L. Grady.

G. REPORT ON UNIT ON PHARMACOLOGY AND PHARMACOKINETICS

This report summarizes the two major projects within this Unit.

I. CLINICAL PHARMACOKINETICS, PHARMACODYNAMICS AND THERAPEUTICS

1. Arecoline treatment of human subjects with Alzheimer's disease. Arecoline was administered intravenously to 19 demented elderly subjects, and plasma pharmacokinetics were measured. Steady-state plasma levels were rapidly achieved and, after termination of the infusion, arecoline was cleared from plasma with a half-life of less than five minutes. Administered by chronic, continuous intravenous infusion, arecoline improved memory performance in

patients with Alzheimer's disease, at doses producing no side-effects. A plasma drug level corresponding to cognitive optimization was identified. Hence, arecoline and related drugs may be effective for palliation of Alzheimer's disease. This work was conducted by T. Soncrant, K. Raffaele, and S. Asthana.

2. Physostigmine treatment of patients with Alzheimer's disease. Physostigmine, a cholinesterase inhibitor, was administered to 9 patients with mild-moderate dementia. Steady-state drug levels were achieved by continuous infusion. Modest cognitive improvement occurred in some subjects; adverse effects were frequent. Specific muscarinic agonists may be more effective than are cholinesterase inhibitors in the palliation of cognitive symptoms of Alzheimer's disease. Conducted by S. Asthana, T. Soncrant, K. Raffaele.

3. Age-related cognitive and psychomotor responses to haloperidol. Young and elderly subjects received haloperidol intravenously. Young subjects showed greater akathisia and sedation, and had higher plasma prolactin levels after haloperidol administration. The results suggest a reduced effect of brain dopamine receptor blockade on cognitive and motor function in aged humans. Conducted by J. Kelly and J. Kaye.

II. CEREBRAL METABOLISM, RELATION TO BRAIN FUNCTION AND AGING

1. Cholinergic function and age. In Fischer-344 rats, aged 3 or 24 months, cerebral metabolic responses to arecoline did not differ, indicating that muscarinic receptor and post-receptor mechanisms are intact in the senescent rat brain. Cortical cholinergic deafferentation by ablation of the nucleus basalis (a possible model of Alzheimer's disease) reduced cortical rCMRglc to a greater extent in 3 month than in 24 month-old rats, likely due to reduced tonic activity of cholinergic presynaptic elements in aged animals. Metabolic responses to cholinergic drugs were not altered by prior nucleus basalis ablation, indicating presynaptic cholinergic reorganization also manifest as altered regional brain functional interactions. This work was conducted by T. Soncrant and E. DeMicheli.

2. Serotonin system and age. Male Fischer-344 rats aged 3, 12, or 24 months were administered the serotonin agonist m-chlorophenylpiperazine (m-CPP) and rCMRglc was measured. Metabolic responses were reduced, but not delayed, in 12 month and further reduced in 24 month, compared to 3 month old, rats despite the achievement of higher brain drug concentrations in the older animals. These results demonstrate presynaptic functional decrements in rat brain serotonergic systems during aging. Changes in regional cerebral metabolic rates for glucose produced by m-CPP are reduced by chronic pretreatment with m-CPP in both young and old rats, indicating that age-related presynaptic functional decrements are adaptive rather than pathologic. This work was conducted by U. Freo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00406-02 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Mechanisms for Alzheimer Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	S. Rapoport	Chief, LN	LN, NIA
OTHERS:	M. Schapiro	Chief, BADS	LN, NIA
	C. Grady	Chief, PET Unit	LN, NIA
	J. Haxby	Chief, Neur. Unit	LN, NIA
	B. Horwitz	Chief, BI&CU	LN, NIA
	N. Gershfeld	Senior Scientist	LPB. NIAMSD
	L. Ginsberg	Visiting Associate	LPP, NIAMSD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Comparative anatomic data suggest that several systems of brain regions underwent selective expansion or differentiation during primate evolution, according to the principle of 'integrated phylogeny'. This involved expansion of the neocortex. Certain human neurodegenerative diseases, including Alzheimer disease, affect such systems, suggesting that they are 'phylogenic' diseases and that the genetic changes that promoted integrated phylogeny are related to the genetics of these diseases. Studies of monozygotic twins discordant and discordant for Alzheimer disease suggest heritable as well as not evidently heritable forms of this disorder, consistent with genetic heterogeneity. Measurements of brain blood flow using positron emission tomography, during cognitive stimulation, suggest that reversible synaptic failure underlies early functional deficits in Alzheimer disease.

The critical temperature for maintaining stable lipid monolayers in vitro is reduced from 37 oC to less than 30 oC, using lipids from temporal association but not cerebellar cortex of Alzheimer brain, suggesting that cell membrane instability in vulnerable brain regions contributes to progression of Alzheimer disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00403-07 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Genetics and Nongenetic Factors in Alzheimer's Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	Mark Schapiro	Chief, Section Bad	LN, NIA
Others:	C. Grady	Chief, PET Unit	LN, NIA
	K. D. Pettigrew	Statistician	MHIRP.M
	Zheng Wu	Visiting Associate	LN, NIA
	Beverly White	Medical Res Officer	LCB, NIDDK
	Katherine K. Sanford	Chief	LCMB, NCI
	Ram Parshad	Professor	Howard Univ

COOPERATING UNITS *(if any)*

CRND Univ., Toronto; Dept. Pathology, Howard Univ.; Twin Registry, NAS; LCNSS, NINDS; MHIRP, NIMH; LCMB, NCI; LCB, NIDDK

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pedigrees were constructed from family histories of patients participating in a dementia program to examine the genetic basis of Alzheimer's disease. Differences in family history between monozygotic twins discordant or concordant for dementia of the Alzheimer type suggest heritable and nonheritable forms of Alzheimer's disease. Experimental protocols were developed for comparing phenotypes and genotypes of individuals with duplications of parts of chromosome 21, to identify genes contributing to mental retardation and dementia. Buffy coats from blood of first degree relatives with dementia of the Alzheimer type were injected into hamsters to test transmissibility in Alzheimer's disease. In animals surviving up to 250 days after inoculation, no evidence of brain disease was present, arguing against transmissibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00140-09 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Cerebrospinal Fluid Chemistry in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	M. B. Schapiro	Chief, SBAD	LN, NIA
OTHERS:	J. R. Atack	Research Scientist	Merck
	I. Hanin	Director	Dept. Pharm, Loyola Univ
	M. F. Beal	Neurologist	Mass Gen. Hosp

COOPERATING UNITS *(if any)*

Dept of Pharmacology and Experimental Therapeutics, Loyola Univ.; Dept of Neurology, MA General Hospital, Boston, Ma; Merck, Sharpe and Dohme, England.

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia/Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In cerebrospinal fluid acetylcholinesterase activity and somatostatin and neuropeptide Y concentrations did not differ from control values in either young or old Down patients. Analysis of lumbar cerebrospinal fluid fractions in healthy young and old subjects indicated no anterior-posterior gradients for somatostatin and neuropeptide Y, but higher acetylcholinesterase in caudal as compared to rostral fractions in younger subjects, indicative of sources for this enzyme from the spinal cord. Cerebrospinal fluid acetylcholinesterase activity was reduced in patients with progressive supranuclear palsy, indicating a central cholinergic deficit. There was no effect on activity of administered physostigmine, and anticholinesterase. Acetylcholinesterase gradients in cerebrospinal fluid were negative in the rostrocaudal direction in young healthy subjects, but were absent in old healthy subjects, who had higher mean activity overall.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00405-06 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

New Investigations in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	M. Schapiro	Chief, BADS	LN, NIA
	C. DeCarli	Senior Staff Fellow	LN, NIA
	D. Murphy	Visiting Associate	LN, NIA
	J. Salerno	Senior Staff Fellow	LN, NIA
	M. Hertzman	Professor Psychiatry	G. W. Univ.
	S. Rapoport	Chief, LN	LN, NIA
OTHERS:	R. Hagerman	Professor of Pediatrics	Univ. of Colorado

COOPERATING UNITS *(if any)*

G. W. Univ., Dept. Psychiatry; School of Medicine, Univ. of Colorado, Laboratory Chemical Biology, NIDDK; Division of Neurology, Dept. of Medicine, Duke Univ.

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia Section

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In using the isotope fluoro-18-deoxyglucose with positron emission tomography, we found that cerebral glucose utilization does not change with advancing age in healthy males, but changes are found in patients with Alzheimer's disease and Down syndrome. Three new protocols were introduced to evaluate these findings and determine their specificity. Studies are underway in multi-infarct dementia, the second leading cause of dementia; major depressive disorder with and without cognitive impairment; and fragile-X syndrome. The role of the dopaminergic system in normal aging, Alzheimer's disease with and without extrapyramidal signs, and familial inverted chorea will be explored with 6-[18-F]-fluoro-L-Dopa and positron emission tomography (PET).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00126-12 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Brain Function in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	M. Schapiro	Chief, SBAD	LN, NIA
	C. Grady	Chief, Unit on PET	LN, NIA
	B. Horwitz	Chief, Unit on Brain Imaging	LN, NIA
	J. Haxby	Chief, Unit on Neuropsy	LN, NIA
	J. Salerno	Medical Staff Fellow	LN, NIA
	D. Murphy	Visiting Associate	LN, NIA
	P. Pietrini	Visiting Associate	LN, NIA
	M. Mentis	Visiting Associate	LN, NIA

COOPERATING UNITS *(if any)*

Child Psychiatry Branch, NIMH, Department of Nuclear Medicine, CC, Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Age-related differences in metabolism and blood flow of frontal cortex were enhanced when young and old subjects received sensory activation. A patient with isolated memory impairment and family history for autosomal dominant AD was studied with PET twice and found to have normal glucose metabolism with routine analysis but both scans were found to be abnormal using a discriminant function analysis. Dorsal vs. ventral patterns of regional cerebral blood flow activation during face and location matching were similar in young and old subjects, but were less functionally distinct in older subjects. Activation of regional cerebral blood flow in occipitotemporal cortex was equivalent during face perception in DAT patients and controls; patients also had frontal activation. Patients with late onset depression had increased brain metabolism in orbitofrontal cortex. A patient with autopsy proven Parkinson's disease had a metabolic pattern indistinguishable from that seen in DAT. Patients with vascular dementia had decreased subcortical glucose metabolism compared to DAT patients. Density of neurofibrillary tangles, but not senile plaques in postmortem brain, was correlated with metabolic reductions. A nonretarded patient with mosaic translocation trisomy 21 had cognitive and metabolic impairments typical of DAT suggesting that genetic determinants of dementia in Down syndrome differ from those responsible for retardation. Patients with trichotillomania, a disease similar to obse ssive-compulsive disorder, had higher glucose metabolic rates than to controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00404-06 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Functional Interactions Among Brain Regions in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	B. Horwitz	Chief, Unit on Brain Imaging & Computers	LN, NIA
	T. Soncrant	Senior Staff Fellow	LN, NIA
	N. Azari	NRC Fellow	LN, NIA
Others:	M. Mentis	Visiting Associate	LN, NIA
	C. L. Grady	Research Psychologist	LN, NIA
	M. B. Schapiro	Chief, BADS	LN, NIA

COOPERATING UNITS *(if any)*
LN, NIMH; CPB, NIMH; DASR, NIMH

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Brain Aging and Dementia

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
A correlation method was developed to examine functional interactions between brain regions, by correlating either regional cerebral metabolic rates for glucose or regional cerebral blood flows, as determined by positron emission tomography (PET) in humans. Women had stronger functional associations than men involving left-hemisphere frontal cortices. There was a loss of frontal-parietal functional associations with age. There was a further reduction in patients with dementia of the Alzheimer type (DAT), suggesting a loss of corticocortical connections. Patients with obsessive-compulsive disorder (OCD) did not show an overall loss of functional associations, but rather a reorganization. A discriminant function involving regional interdependencies could distinguish DAT from controls.

In humans in whom regional cerebral blood flow (rCBF) was measured with PET during two visual processing tasks, correlations among visual brain areaas were significant on the right side, but not the left side, suggesting a more important role for the right hemisphere.

In awake Fischer-344 rats, having undergone corpus callosotomy, reduced metabolic correlations were found between left and right hemispheric brain regions, suggesting that interhemispheric interactions are mediated in part by callosal fibers. Correlations between cholinergic nuclei and frontoparietal cortex increased in rats 2 weeks after a lesion of the nucleus basalis magnocellularis.

A network-simulation model was developed to partially validate the correlational analysis as applied to blood flow data, and to explore the neurobiological substrates of observed correlational patterns.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00129-12 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Regulation of Brain Nutrients & Metals in Aging & Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	Q. R. Smith	Chief, SNBT	LN, NIA
	K. Washizaki	Visiting Fellow	LN, NIA
	Y. Kohmo	Visiting Fellow	LN, NIA
	Q. Deng	Visiting Associate	Nanjing, China
Others:	S. I. Rapoport	Chief, LN	LN, NIA
	N. H. Greig	Pharmacologist	LN, NIA
	K. C. Wadhwani	Senior Staff Fellow	LN, NIA

COOPERATING UNITS *(if any)*

Unit on Neurotoxicology, INSERM, Paris,France; Intramural Research Program, NINDS; Section on Analytical Biochemistry, NIMH; Biomedical Mass Spectrometry Unit, University of New South Wales; Tokyo Medical and Dental University, Tokyo, Japan;

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Neurochemistry and Brain Transport

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The basic amino acid transporter of the blood-brain barrier was identified and shown to be homologous to the cell surface receptor for ecotropic murine retroviruses. Transporter mRNA expression was greatest in brain capillaries and exceeded that in parenchymal brain by >10 fold. The structural selectivity of the cerebrovascular large neutral amino acid carrier was characterized and used to design drugs showing rapid transport into brain. One such drug, D,L-NAM, was shown to have the highest affinity of any known agent for the transporter and to be taken up into brain 20 times more rapidly than its clinical analogue, melphalan. The pharmacokinetics of beta-N-methylamino-L-alanine (BMAA), a neurotoxin that has been implicated in the pathogenesis of amyotrophic lateral sclerosis and parkinsonism dementia in the western Pacific, was examined in rats. BMAA was found to reach toxic concentrations in brain following high dose administration and was transported into brain by the large neutral amino acid carrier of the blood-brain barrier.

Saturable carriers were identified at the blood-brain barrier for manganese, calcium and chloride. The brain uptake and deposition of toxic metals, including aluminum, lead, cadmium and manganese, was enhanced by calcium deficiency, stressing the importance of diet in brain metal toxicity. Low dietary calcium also produced a precipitous drop (>90%) in serum copper and created imbalances in other essential brain metals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AG 00130-09 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neuropsychological Function in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	J.V. Haxby	Unit Chief, Neuropsychology	LN, NIA
	C.L. Grady	Research Psychologist	LN, NIA
Others:	K. Raffaele	Staff Fellow	LN, NIA
	J. Maisog	Staff Fellow	LN, NIA
	M. Mishkin	Chief, LNP	LNP, NIMH
	L.G. Ungerleider	Research Psychologist	LNP, NIMH
	R. Parasuraman	Cognitive Psychologist	Catholic U

COOPERATING UNITS *(if any)*
Laboratory of Neuropsychology, NIMH Department of Psychology, Catholic University

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Brain Aging and Dementia

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The presence and neuroanatomical location of separate visual pathways for spatial vision and object vision were investigated in healthy young men by measuring regional cerebral blood flow (rCBF) with positron emission tomography (PET) and $H_2{}^{15}O$. The results identified extrastriate visual areas associated with these pathways: dorsolateral occipital and superior parietal areas activated more by spatial visual processing, and ventral occipital and occipitotemporal areas activated more by object discrimination. Old subjects demonstrated rCBF activations in the same regions as did young subjects, but also demonstrated activation of ventral areas during spatial vision and dorsal areas during object vision, suggesting less functional separation of these visual systems. Patients with dementia of the Alzheimer type (DAT) demonstrated normal percent baseline activation of rCBF in occipitotemporal cortex during object vision, suggesting preserved capacity to recruit this area for perceptual processing. In healthy adults, significant age-related differences were found on tests of perceptual reasoning and visual memory, but not on verbal memory and verbal intelligence. Age-related visual memory differences could be accounted for by the age-related differences in visual cognitive processing, suggesting that they are restricted to visuospatial performance with relative preservation of memory functions. Neuropsychological decline in very early Alzheimer's disease was found to have an early plateau phase followed by steady, linear decline. Rate of cognitive decline is significantly correlated with rate of brain tissue loss, as measured with serial CT scans, and with rate of worsening abnormality of resting state regional cerebral metabolic rates for glucose (rCMRglc) as measured by PET and ^{18}F-Fluorodeoxyglucose. Impairment of complex attention, the first nonmemory function to demonstrate impairment in early DAT, was investigated with cognitive studies which identified three similar impairments of shifting or divided attention. Older Down syndrome adults perform worse on mental abilities tests than do younger subjects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00133-10 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Clinical Pharmacokinetics, Pharmacodynamics and Therapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	T. Soncrant	Medical Officer	LN, NIA
	S. Asthana	Visiting Associate	LN, NIA
	J. Kelly	NRC Fellow	LN, NIA
	K. Raffaele	Staff Fellow	LN, NIA
	J. Haxby	Staff Psychologist	LN, NIA
Others:	N. Greig	Visiting Scientist	LN, NIA
	A. Berardi	Guest Researcher	LN, NIA

COOPERATING UNITS *(if any)*

College of Pharmacy, University of Saskatchewan Bristol Myers-Squibb Co., Wallingford, CT

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Arecoline, a cholinergic agonist, improves memory in patients with Alzheimer's disease when given by continuous intravenous infusion. The degree of improvement follows an inverted U-shaped relation to dose, and is maximal at a dose at least ten-fold below that producing toxicity. A plasma concentration was identified that achieves maximal cognitive improvement in responding patients. Physostigmine, a cholinesterase inhibitor, given by continuous intravenous infusion improved cognition in some subjects with Alzheimer's disease, but was associated with many adverse effects. Administration of haloperidol, a dopamine antagonist, produced greater cognitive and motor effects in young than in aged healthy men, suggesting that responsivity of the brain dopamine system is reduced with age in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00128-12 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Analytical Drug Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	T. Soncrant	Medical Officer	LN, NIA
	L. Hegedus	Visiting Associate	LN, NIA
	J. Deutsch	Visiting Scientist	LN, NIA
	N. Greig	Visiting Scientist	LN, NIA
Others:	H. Holloway	Biologist	LN, NIA

COOPERATING UNITS *(if any)*

Hebrew University of Jerusalem

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An analytical method using high performance liquid chromatography (HPLC) with fluorescence detection was developed for the measurement of physostigmine, a cholinesterase inhibitor, in plasma of humans receiving the drug as a treatment for Alzheimer's disease. A gas chromatographic/mass spectrometric assay for the measurement of nicotine in rat plasma and brain and in human plasma was developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00125-14 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Cerebral Metabolism, Relation to Brain Function and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	T. Soncrant	Medical Officer	LN, NIA
	E. De Micheli	Visiting Fellow	LN, NIA
Others:	H. Holloway	Biologist	LN, NIA
	D. Larson	Biologist	LN, NIA
	N. Greig	Visiting Scientist	LN, NIA
	U. Freo	Visiting Fellow	LN, NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Changes in regional cerebral metabolic rates for glucose produced by the serotonin agonist m-chlorophenylpiperazine (m-CPP) are reduced by chronic pretreatment with m-CPP in both young and old rats, demonstrating preservation of serotonin regulation during aging. The metabolic effects of m-CPP depend upon adrenal function. In a model of cholinergic cortical deafferentation, produced by lesioning the nucleus basalis magnocellularis cerebral metabolic deficits were smaller in aged rats, demonstrating an age-related reduction in tonic cholinergic input to the neocortex. Metabolic responses to cholinergic drugs were preserved in this model, indicating presynaptic reorganization also manifest as altered regional brain functional interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00407-01 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Neuroanatomy and Neuropathology of Aging Primate Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	D. Brady	Senior Staff Fellow	LN, NIA
Others:	J. Stoll	Senior Staff Fellow	LN, NIA
	K. Chandrasekaran	Visiting Scientist	LN, NIA
	A. Balbo	Biologist	LN, NIA
	E. Mufson	Professor	Rush Med
	S. Zain	Professor	U. Roches.

COOPERATING UNITS *(if any)*

Division of Neurosciences, Rush-St. Lukes Medical Center, Chicago, IL; Univ. of Rochester, Rochester, NY

LAB/BRANCH

Laboratory of Neurosciences

SECTION

None

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The topographic distribution, morphology and pattern of fiber staining of nicotinamide adenine dinucleotide-diaphorase (NADPH-d) containing structures was shown to be largely similar in the amygdala of the squirrel monkey and normal aged human. NADPH-d cells are resistant to neurodegeneration in Huntington's disease and may be resistant to neurodegeneration in Alzheimer's disease (AD). The selective vulnerability hypothesis was tested in the hippocampal formation and amygdala in patients with AD, demonstrating up to a 50% decrease in neurons containing the calcium-buffering protein, parvalbumin. Those areas connectionally associated with the cerebral cortex, e.g., the dentate gyrus, CA1 and lateral nucleus of the amygdala, showed the significant declines in the number and morphologic diversity of parvalbumin neurons. This suggests that connectivity of a specific cytoarchitectural area features in the pathogenesis of AD. A molecular biology approach toward understanding the role of long projection neurons was undertaken using cDNA clones derived by differential hybridization. This revealed cytochrome oxidase (COX) mRNA was differentially expressed in long projection neurons as compared to small interneurons. Moreover, COX enzyme activity was differentially expressed in terminal fields of long projection systems, e.g., the perforant pathway in the hippocampal formation. A major goal of many research labs is the generation of a useful animal model for AD. We evaluated a transgenic mouse model whose transgene contained the carboxy-terminal 100 amino acids of the amyloid precursor protein. There was no evidence of increased beta-amyloid protein expression in murine brain, nor any neuropathologic changes even remotely similar to AD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00135-09 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Molecular Biology of Brain Aging and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	K.Chandrasekaran	Visiting Scientist	LN, NIA
	J.Stoll	Senior Staff Fellow	LN, NIA
	R.Fukuyama	Visiting Associate	LN, NIA
Others:	A. Fine	Scientist	Dalhousie Univ.
	B. Ault	Scientist	Sterling Drug Co.
	A. Balbo	Biologist	LN, NIA
	N. Lane	Scientist	Univ. Cambridge.

COOPERATING UNITS *(if any)*
Dept. Physiology, Dalhousie Univ., Halifax, NS: Sterling Drug Co., Rensselaer, NY; Univ of Cambridge, England

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	2.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
A frontal pole cDNA library from the rhesus monkey brain was shown to have 6 cDNA clones which are differentially expressed as compared with primary sensory cortices. Three of these clones were identified as mitochondrial DNA encoded genes, cytochrome oxidase (COX) subunits I. II and III, reflecting greater neuropil (synapses and dendrites) and mitochondrial density in association as compared to primary cortices. Such differences in gene expression may be related to selective vulnerability of association than of primary cortical regions in Alzheimer's disease (AD) in humans. Localization of COX activity and mRNA also identified the neurons that are involved in pathway specific and corticocortical connections in monkey and human brain. This distribution of COX mRNA corresponded with the neurons that are selectively vulnerable in Alzheimer's disease. Mouse trisomy 16 (ts16), an animal model for Down syndrome in humans, is a lethal chromosomal abnormalitiy leading to fetal death. However, Ts16 hippocampal tissue was successfully transplanted into brains of normal mouse hosts and survived for periods up to 18 months. The grafts did not demonstrate evidence of Alzheimer's disease neuropathology, suggesting that increased expression of the genes homologous to those on human chromosome 21 is insufficient to cause Alzheimer type neurodegeneration in the mouse.

Others:

D. Brady	Senior Staff Fellow	LN, NIA
S. Rapoport	Chief	LN, NIA

Project description:

Objectives:

Molecular biology has provided a powerful way to isolate and characterize genes and to manipulate their expression. This approach can be used to understand the molecular basis of cellular aging, particularly in an organ as complex and highly evolved as the brain. The objectives of this research are 1) to determine if there are quantitative or qualitative differences in gene expression in regions of brain susceptible to Alzheimer's disease, using a nonhuman primate model and human brain samples; 2) to analyze expression of particular genes believed to be responsible for the phenotypes of Down syndrome and Alzheimer's disease; and 3) to analyze expression of various genes on human chromosome 21 and on mouse chromosome 16, in the trisomy 16 mouse, an animal model of Down syndrome (see Ann. Rep. Z01 AG 00407-01 LN).

Methods Employed:

1. Identification of region-specific mRNAs in nonhuman primate brain. Brains of Macaca mulatta monkeys were removed, specific regions were dissected and mRNA were isolated. cDNA libraries were generated from mRNA in both lambda gt10 and gt11 vectors. cDNA clones were screened by differential hybridization. Clones that hybridized with the probe prepared from one region but not with the probe from another region were isolated and characterized.

2. COX enzyme activity and COX mRNA were localized by diaminobenzidine histochemistry and in situ hybridization respectively.

3. APP biosynthesis, localization and the level after chemical treatment in PC12 cells and its variant PC12S cells grown with or without NGF were monitored by immunofluorescent staining, Western blotting and immunoprecipitation procedures.

4. Hippocampal regions of developing rat embryo brain were used to immunize BALB/c mice in order to produce monoclonal antibodies. Myeloma cells and lymphocytes of immunized mice were fused and hybridomas were selected. Immunofluorescent staining of embryo and adult rat brains was carried out to screen hybridomas secreting antibodies.

5. Hippocampus was dissected from gestation day 15-17 mouse fetuses, either trisomic for chromosome 16 or normal littermate controls. Tissue was grafted into the striatum or ventricles of young female mice (6-8 weeks) as a chunk or as individual cells dissociated by trypsin. The mice were allowed to survive for up to 2 years and then the grafts were examined for expression of genes by in situ hybridization, Northern blotting and RNAse protection and for the appearance of Alzheimer's pathology by histochemistry and immunocytochemistry.

Major Findings:

1. Five cDNA libraries from various monkey brain regions were constructed in two vectors: lambda gt10 and gt11. Screening of the libraries by differential hybridization revealed a number of clones that hybridized with the probe from the frontal pole but not with the probe from the primary visual cortex. A secondary screening and Northern blot analysis confirmed the differential expression of these genes. Characterization of some of these clones showed identity to mitochondrially encoded cytochrome oxidase (COX) genes (Chandrasekaran, K., Stoll, J., Giordano, T., Atack, J., Matocha, M., Brady,

D.R. and Rapoport, S.I., Differential expression of cytochrome oxidase (COX) genes in different regions of monkey brain, J Neurosci Res, 1992;32(3):415-423).

2. Within the monkey hippocampal formation, the terminal fields of the perforant pathway showed the highest levels of COX activity, whereas COX mRNA was localized mainly in neuronal cell bodies. In the entorhinal cortex, COX mRNA was detected in neuronal cell bodies of layers II and IV. Localization of COX and its mRNA correlated with the input and output pathways of the hippocampus (Chandrasekaran, K., Stoll, J., Brady, D.R. and Rapoport, S.I., Localization of cytochrome oxidase (COX) activity and COX mRNA in the hippocampus and entorhinal cortex in the monkey brain: Correlation with specific neuronal pathways. Brain Res 1992;579:333-336).

3. The analysis on localization of COX mRNA and enzyme activity was extended to include other areas of the cerebral cortex. Prorhinal, perirhinal, superior temporal association and lateral frontal cortices were evaluated. In all these areas, highest levels of COX mRNA was localized to cellular layers containing intra and interhemispheric projection neurons, whereas, COX activity was highest in synapse rich layers, e.g, layer I. (Chandrasekaran, K., Stoll, J., Rapoport, S.I. and Brady, D.R., Localization of cytochrome oxidase (COX) activity and COX mRNA in the perirhinal and superior temporal sulci of the monkey brain. Brain Research, submitted, 1992).

4. These findings are relevant to Alzheimer's disease, since the long projection neurons are susceptible to neurodegeneration. Correspondingly in AD brains, a decrease in COX mRNA was detected in these vulnerable neurons (Chandrasekaran, K., Stoll, J., Brady, D.R. and Rapoport, S.I., Distribution of cytochrome oxidase (COX) activity and mRNA in monkey and human brain. COX mRNA distribution correlates with neurons vulnerable to Alzheimer's pathology. Soc. Neurosci. Abstr., 1992).

5. APP is localized to the cytoplasm of PC12 and PC12S cells before differentiation by nerve growth factor (NGF). After NGF treatment, APP is localized to growth cones of neurites in PC12S cells but not in PC12 cells. Western blotting and immunoprecipitation demonstrated selective induction of synthesis of APP695 after neuronal differentiation of PC12 cells. Addition of agents which lead to a perturbation in the levels of ATP, or of calcium, showed decrease in APP and synaptophysin levels. (Fukuyama,R., Chandrasekaran, K., Galdzicki, Z., Stoll, J. and Rapoport, S.I. Alteration in the level of amyloid precursor protein (APP) by chemical treatment in cultured cells: a probe to study the possible cellular function of APP, Abstract, Third International Conference on Alzheimer's Disease & Related Disorders, 1992).

6. Immunofluorescent staining with culture media of hybridomas demonstrated stains of 1) neuron, 2) glia, 3) connective tissue, 4) choroid plexus and 5) other structures. One monoclonal antibody specifically stains granule and pyramidal neurons of hippocampus, granule and Purkinje neurons of cerebellum and some but not all neurons of cortex and reacts with several protein antigens expressed in PC12 cells. Another monoclonal antibody specifically labels glia and choroid plexus. The antibody recognizes a 30 kDa protein expressed only in C6 glioma cells (Fukuyama and Rapoport, "Isolation of monoclonal antibodies against hippocampus of developing rat brain", being presented in 22nd Annual Meeting Society for Neuroscience, 1992).

7. Hippocampal tissue from embryonic day 15-17 fetal mice, trisomic for chromosome 16 or euploid littermates, was transplanted into the striatum or the lateral ventricle of 6-8 week old female C57Bl/6 mice. After six to fourteen months of survival, host brains were sectioned and the grafts were examined by standard histochemical techniques or by immunocytochemistry for antigens present in pathological brain of Alzheimer's disease (AD) patients. Nissl-stained grafts contained aggregations of neurons similar to the

pyramidal- or the granule-cell layers of the normal adult hippocampus. No obvious morphological difference was detected between trisomic and control transplants. The monoclonal antibody Alz-50, which recognizes paired helical filaments characteristic of AD, and an antibody raised to beta-amyloid peptide, did not reveal neurodegeneration in these grafts. Antibodies against ubiquitin, 200 kDa subunit of neurofilament, alpha-1-antichymotrypsin and tau also did not demonstrate AD-type pathology in the trisomic or control grafts. Examination of thioflavine-S or silver stained sections were also negative. We conclude that the trisomy 16 mouse is not a model for AD-type neurodegeneration. These results differ from those of Richards et al., EMBO J. (1991) 10:297-303, who reported AD-type degeneration in trisomy 16 transplants. We have no explanation for their positive findings, except to suggest that they may refer to an occasional degenerating neuron in their grafts.

Gene expression in the grafts was qualitatively the same as the host hippocampus as assessed by in situ hybridization using probes for amyloid precursor protein, somatostatin, GAP-43, MAP-2. Quantitative assessment is currently underway by performing RNAse protection and Northern blot analysis on RNA isolated from dissected grafts. Abstracts of this work have been presented (Stoll et al., Examination of Alzheimer-type pathology in mouse trisomy 16 neurons maintained by transplantation, Soc. for Neurosci. (1991) abstract 421.2; Stoll et al., examination of Alzheimer-type neurodegeneration in mouse trisomy 16 neurons maintained by transplantation, IVth International Symposium on Neural Transplantation (1992)).

Significance to Biomedical Research and the Program of the Institute:

1. Alzheimer's disease is a neurodegenerative disorder that predominantly affects the association neocortices and connected regions. Characterization of genes that are specifically expressed in these regions is a rational approach to understand why this disease affects higher order cognitive functions. The results of this research will provide novel well-defined DNA probes with which to address the molecular basis of Alzheimer's disease.

2. Clinical observations and postmortem pathology studies indicate that the occurrence of Alzheimer's disease in aged Down syndrome subjects approaches 100%. Whether all of the genes mapped to chromosome 21 have a role in the onset of AD is not known. An understanding of the gene dosage effect in Down syndrome, i.e., if all of the loci are coordinately overexpressed, should provide important information on which gene(s) may have a role in the onset of Alzheimer's disease in these individuals.

3. The mouse chromosome 16 is partially syntenic to human chromosome 21. Quantitation of gene expression in this mouse model will provide information on the effect of gene dosage on RNA transcript levels in the developing brain.

Proposed Course:

The current focus of this program is on identifying region-specific mRNAs and proteins in the nonhuman primate brain. The cDNA clones already identified will be utilized by in situ hybridization to evaluate expression in various dissected brain regions. The role of mitochondrial oxidative metabolism in neurodegeneration will be analyzed. Additional approaches are being planned to understand the molecular basis of Alzheimer's disease. These include characterization of obligate Down syndrome region in terms of developing transcriptional maps and comparison of gene expression between normal and Alzheimer brains. The mouse trisomy 16 grafts will be used to study the effects of aneuploidy on gene expression.

Publications:

1. Chandrasekaran K, Stoll J, Brady DR, Rapoport SI: Localization of cytochrome oxidase (COX) activity and COX mRNA in the hippocampus and entorhinal cortex of the monkey brain: correlation with specific neuronal pathways. Brain Res 1992;579:333-336.

2. Chandrasekaran K, Stoll J, Giordano T, Atack JR, Matocha MF, Brady DR, Rapoport SI: Differential expression of cytochrome oxidase (COX) genes in different regions of monkey brain. J Neurosci Res 1992;32(3):415-423.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00132-08 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Brain Anatomy in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	C. DeCarli	Senior Staff Fellow	LN, NIA
	B. Horwitz	Chief, Unit on Brain Imaging & Computers	LN, NIA
	D. Murphy	Visiting Scientist	LN, NIA
Others:	M. Schapiro	Chief, BADS	LN, NIA
	S.I. Rapoport	Chief	LN, NIA
	D. Teichberg	Computer Programer Analyst	LN, NIA

COOPERATING UNITS *(if any)*
LNC, NIMH; NIS & DIR, NINDS; CC Radiology; General Electric Co., Schnectady, NY.

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Brain Aging and Dementia

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☐ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Quantitative X-ray computer assisted tomography (CT) analyses in healthy aging men and women demonstrated significant sex differences in lateral ventricular volume and in age of onset of lateral ventricular enlargement. Longitudinal analysis of serial CT scans showed that the rate of ventricular dilation prior to the onset on nonmemory cognitive deficits in patients with dementia of the Alzheimer type (DAT) is less than after the onset of these deficits.

Age-related subcortical gray matter atrophy, ventricular dilatation, and the expansion of peripheral cerebrospinal fluid (CSF) volume was found in healthy men using magnetic resonance imaging (MRI). MRI showed a significant age-related decrease of posterior frontal, but not of temporal lobe volume in healthy men. Men with mild DAT had significantly less brain matter than did healthy age-matched controls.

Mean volumes of both the right and left lateral ventricles were significantly larger in the neurologically and cognitively normal hypertensives as compared to controls using MRI. Whereas enlargement of the lateral ventricles appears to be a concomitant of normal aging, our findings suggest that longstanding hypertension results in greater ventricular enlargement than would be expected on the basis of age alone.

^{31}P spectra, and fluorodeoxyglucose positron emission tomographic (PET) scans were obtained from DAT patients and healthy age-matched controls. Phosphorus metabolite concentrations or ratios did not differ between patients and controls. Glucose metabolic rates in the same volume of brain were significantly reduced in DAT patients relative to controls. These results suggest that disturbances in cellular phosphate energy reserves and membrane phosphoester metabolite levels do not play a major role in the neuropathology of DAT, at least in bulk regions of the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00120-15 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Blood-Brain Barrier and Central Nervous System Function.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI	N. H. Greig	Visiting Scientist	LN, NIA
Others:	S. I. Rapoport	Chief	LN, NIA
	T. Soncrant	Unit Chief	LN, NIA
	R. Rothman	Chief	LCS, NIDA
	A. Brossi	Scientist Emeritus	NIDDK
	K. Wozniak	Visiting Scientist	LCS, NIAAA
	D. Ingram	Unit Chief	LCMB, NIA

COOPERATING UNITS *(if any)*

LCS, NIDA, LC, NIDDK, LCS, NIAAA, LCMB, GRC/NIA

Dept. Expl. Pediatrics, M.D. Anderson Cancer Center, Houston, TX

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The factors that co-determine the amount of drug that enters and is maintained in brain following its systemic administration were analyzed to aide in the safer use of drugs in the elderly and the development of central nervous system therapeutics. Rationale strategies were developed to design selective, long-duration and centrally active cholinesterase inhibitors for the palliative treatment of Alzheimer's disease and agents also were developed to improve the treatment of malignant brain tumors. Specifically, novel analogues of physostigmine were developed as cognitive enhancers and a novel lipophilic anticancer alkylating agent, a tertiary butyl ester of chlorambucil, was developed for brain tumor treatment. Further, therapeutics and strategies are being developed for the treatment of neurologic diseases, of drug abuse and to improve drug delivery to brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00132-08 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neuronal Development in Tissue Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	Z. Galdzicki	Visiting Associate	LN, NIA
	E. Coan	Visiting Fellow	LN, NIA
Others:	A. Balbo	Biologist	LN, NIA
	P. Caviedes	Assistant Professor	U. Chile
	D. Gambal	Professor	Creighton Univ.

COOPERATING UNITS *(if any)*
Department of Physiology, University of Chile, Santiago, Chile.
Department of Biochemistry, Creighton Univ, Omaha, Neb.

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0		

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Studies using cultured hippocampal neurons from fetal mouse trisomy 16 (Ts16), a model for Down syndrome showed that there is a significant decrease in the rate of depolarization of the action potential, and in the voltage-dependent sodium current for trisomy 16 compared to control hippocampal neurons. High voltage-activated calcium currents however were significantly larger in trisomy neurons compared to control.

Withdrawing nerve growth factor (NGF) from the culture medium of control dorsal root ganglion (DRG) neurons resulted in a smaller potassium current compared to those maintained in NGF-containing medium. Trisomy 16 DRG neurons grown in medium that had NGF withdrawn however displayed a larger potassium current. The depolarization rate and sodium current was unaffected in either control or trisomy neurons. Control and trisomy 21 DRG neurons grown in the absence of NGF displayed prolonged action potentials with a slower rate of depolarization. Trisomy 16 DRG neurons in culture had a 2-fold greater incorporation of [1-14C]arachidonate than control neurons, primarily into a lipid resembling phosphatidic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00134-09 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Brain Phospholipid Metabolism, Relation to Function and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	D. Purdon	Special Expert	LN, NIA
	W. Williams	NRC Associate	LN, NIA
	M. Chang	Senior Staff Fellow	LN, NIA
	N. Appel	Guest Scientist	LN, NIA
OTHERS:	N. Appel	Guest Scientist	FDA
	J. Bell	Chemist	LN, NIA
	L. Freed	Guest Worker	LN, NIA

COOPERATING UNITS *(if any)*

Dept. of NUclear Medicine, Clinical Center NIH; Food and Drug Administration.

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using radiolabeled free fatty acids, a mathematical model was developed to calculate transfer rate constants and rates of incorporation from plasma of plasma free fatty acid (FFA) into individual brain regions and brain phospholipids. Radiolabeled palmitic acid (PA, e.g. 3H-PA) is incorporated into the sn-1 of phosphatidylcholine; [3H]-arachidonic acid (3H-AA) is incorporated into the sn-2 position of phosphatidylinositol and phosphatidylcholine, while radiolabeled docosahexaenoic acid (DHA) is incorporated into the sn-2 position of phosphatidylethanolamine. [1-14C]arachidonate was used in awake rats, with or without cholinergic stimulation to monitor the effect of lesioning of the nucleus basalis magnocellularis. We demonstrated an increased uptake of radiolabeled fatty acids in tumor cells relative to surrounding brain parenchyma. Administration of serotonergic agonists increased brain incorporation of 14C-AA but not 3H-PA. This again demonstrates the ability of our approach (e.g. 3H-AA) to study second messenger systems, in vivo. We have now synthesized C-11 radiolabeled PA and AA for use in imaging monkey brain phospholipids by positron emission tomography (PET) and PET studies using these compounds in monkey have been initiated with positive results. Biochemical analysis and modeling of phospholipid metabolism using radiolabeled fatty acids to monitor the transfer of fatty acid into brain phospholipid has also been further analyzed both in brain perfusion and blood infusion models while paying particular attention to the incorporation of fatty acid into phospholipid precursor pools i.e. brain free fatty acid and brain acyl-Coenzyme A. We are now in the last stage of determining parameters for our fatty acid mathematical model. A complete analysis of brain capillary phospholipids and its variation with age has also been completed. Work on endogenous phospholipase A2 and its pharmacological inhibition has been initiated in vitro and will be extended to the brain perfusion model (i.e. in vivo).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00121-15 LN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*

Function and Structure of Peripheral Nerve

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	K. C. Wadhwani	Senior Staff Fellow	LN, NIA
	Q. R. Smith	Section Chief	LN, NIA
Others:	S. I. Rapoport	Laboratory Chief	LN, NIA
	J. Koistinaho	Visiting Fellow	LN, NIA
	C. Latker	Senior Staff Fellow	LN, NIA
	A. Balbo	Technician	LN, NIA
	V. Murphy	Senior Staff Fellow	LN, NIA

COOPERATING UNITS *(if any)*

US Uniformed Health Services, MD

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Neurochemistry and Brain Transport

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Blood-nerve barrier permeability to ions and nonelectrolytes is very low, indicating limited exchange between plasma and nerve endoneurium. The nerve barrier, unlike the blood-brain barrier, does not have a regulatory transport system for calcium. The integrity of the blood-nerve barrier to small nonelectrolytes is maintained with age in the rat. The blood-nerve barrier has regulated carriers for the transfer of manganese , neutral amino acids, and basic amino acids between plasma and nerve. In addition, cationized albumin is taken up into nerve at a greater rate than native albumin, possibly by receptor-mediated transcytosis. Permeabilities of both nerve capillaries and perineurium increase during the first few weeks of Wallerian degeneration. Nerve capillary permeability eventually returns to normal, but perineurial permeability remains elevated, suggesting that nerve fibers are required to maintain perineurial nerve barrier integrity.

ANNUAL REPORT
OF THE
EPIDEMIOLOGY, DEMOGRAPHY, AND BIOMETRY PROGRAM
NATIONAL INSTITUTE ON AGING

Overview

The Epidemiology, Demography, and Biometry Program (EDBP) (1) Plans, conducts, and directs epidemiology, demography, and biometry programs relevant to the mission of the National Institute on Aging; (2) collects and analyzes data regarding the distribution of the aged by such categories as sex, race, socioeconomic and demographic characteristics and serves as a focal point for these data; (3) plans, initiates, coordinates, and analyzes national and international epidemiologic longitudinal studies and studies of special populations; (4) in collaboration with NIA staff and that of other Institutes, federal agencies and scientific organizations, recommends priorities and develops epidemiologic studies of specific diseases and conditions affecting the aged; (5) provides consultation and service to NIH program areas and private organizations on epidemiology, demography, and biometry studies on aging; (6) recommends mechanisms to be used or develops mechanisms to accomplish Program objectives; (7) plans and directs research and training in the areas of epidemiology, demography, and biometry, and serves as the primary federal source of information regarding research and training in these areas.

Accomplishments during fiscal year 1992 were consistent with this mission statement. Various administrative and analytic activities are presented in detail in the sections from the Epidemiology and Demography Office, Asia-Pacific Office, Geriatric Epidemiology Office, and the Biometry Office. Scientific and technical publications covering work done in the EDBP are also presented in the NIH Scientific Directory and Annual Bibliography as well as the NIA/EDBP Sourcebook. In particular, EDBP staff continued supervision of data analytic and collection activities at the four Established Populations for Epidemiologic Studies of the Elderly (EPESE) sites including the sixth year in-person follow-up contact in North Carolina with the addition of an immunology component. A particularly noteworthy programmatic achievement is the preparation of baseline data for archiving. Also, immunologic studies have been added to the Framingham Heart Study. The Women's Health and Aging Study, a major investigation of disease and disability with the John Hopkins University School of Medicine completed plans to initiate data collection in October 1992. Evaluations for presence of dementia began as part of the Honolulu Heart Program cohort examination cycle in March 1991 and have continued to the present. This is a segment of a larger cross-cultural study involving Japan and other sites.

The EDBP continued to be guided by three longer term research themes which are: Aging Processes and Conditions; Dementia, Disease and Disability; and Medical and Long-Term Care. After further consideration of a larger list of alternatives by Ad Hoc reviewers and within the National Institute on Aging the following initiatives are being implemented: Mortality in Centenarians, Head Injury and Alzheimer's Disease, and Cancer and Co-

Morbidity. The pilot work for the Head Injury and Alzheimer's Disease initiative, however, has progressed more slowly than expected, and the onset of the full study will likely have to be delayed until early in fiscal year 1993.

Enhancements to the Honolulu Dementia Study on communication-magnetic resonance imaging and fat free mass-disability are proposed for fiscal year 1993. These initiatives are consistent with the EDBP commitment to studies directed at "Dementia, Disease, and Disability." They are also consistent with the high priority areas listed in the draft NIH Strategic Plan for Aging and for Chronic and Recurrent Illnesses and Rehabilitation. Specifically, they pertain to the areas of Alzheimer's Disease and Related Dementias of Aging; Visual, Hearing and other Sensory and Communicative Loss; and Mobility. A continuation of studies directed at elucidating the effect of co-morbidity on cancer diagnosis and treatment is planned for fiscal year 1993 (see below).

In order to have the capacity to continue current programs and develop new areas of research reorganization of the EDBP was completed with the consolidation of demography and epidemiology functions in a single office. Dr. Jack Guralnik is the Chief of this office. This organizational change allows better integration of epidemiologic issues with "medical" demography such as refinement of disease and impairment estimates, better definitions of disability, frailty measurement issues, and assessment of the impact of medical technology on health status. The Geriatric Epidemiology Office plans and conduct studies and analysis of data in the areas of biomedical, genetic, metabolic, and gerontological issues. Dr. Tamara Harris is the Chief of this office.

The EDBP continues a major training function. Over the next 2 years we will be supporting two outstanding PHS Epidemiology Training Program fellows (Drs. Lori Brown and John Sorkin). In addition, Dr. Luigi Ferrucci, a geriatrician from Florence, Italy, was a Visiting Associate for 9 months. Recently, Dr. Marco Pahor, a geriatrician from Rome, Italy, joined the Program as a guest researcher. EDBP is planning to establish a separate salary resource for visiting foreign scientists similar to the Gerontology Research Center.

Because of a hiring freeze the Program has had to curtail recruitment activities for a statistician for the Biometry Office, an epidemiologist for the Geriatric Epidemiology Office, and a demographer for the Epidemiology and Demography Office. Dr. Marian Parrott has joined the staff as a part-time geriatrician through the Intergovernmental Personnel Act (IPA). Ultimately, these personnel additions will round out an already committed staff and allow the Program to continue to develop more hypothesis-driven research in the fulfillment of the missions of the Program and the Institute.

Dr. Richard J. Havlik continued as Associate Director, EDBP and is a member and currently chairperson of the PHS Epidemiology Training Program Steering Committee; a member of the NIH Epidemiology Coordinating Committee and the Biomedical Scientific Research Service Credentialing Committee. Dr. Havlik is the NIH representative to the National Death Index Advisory Committee. He gave presentations to the Epidemiology of Aging course at Johns Hopkins School of Hygiene and Public Health and the Arlie House conference on Research in Aging; and, he presented research results on vitality and aging at

the 1991 Symposium on the International Collaborative Effort on Measuring the Health and Health Care of the Aging. He participated on the search committee for the Chief, Epidemiology and Data System Branch, National Institute on Deafness and other Communication Disorders.

Cancer in the Aged

1. Comorbidity and Early Diagnosis of Cancer in the Elderly

The National Institute on Aging (NIA) and the National Cancer Institute (NCI) have initiated a collaborative study on cancer in the elderly using the resources of the NIA Epidemiology, Demography, and Biometry (EDB) Program and the NCI Surveillance, Epidemiology, and End Results (SEER) Program. The NIA project officers for the NIA/NCI SEER Collaborative Study are Drs. Rosemary Yancik and Richard Havlik. The study began in October 1991. Initial months were devoted to organizing the study infrastructure and developing instrumentation. After pretesting several instruments designed to collect information from medical records to assess concurrent medical conditions and physical functioning, Phase I, a pilot study, began in April 1992 in five geographic SEER sites -- Iowa; Utah; New Mexico; Seattle, Washington; and San Francisco-Oakland, California. The objective of the pilot study is to ascertain the feasibility of developing comorbidity profiles of older-aged persons who have been diagnosed with cancer.

The pilot study is addressing the practical issues of the implementation of the major study. Aside from staffing the study and fitting the fieldwork into the SEER Program framework, administering questionnaires to recently diagnosed and treated cancer patients may present complex problems (e.g., access, patient consent, interaction with caregiver). The pilot study will identify and characterize conducting such research to develop information for optimum approaches to the cancer patient respondent. Phase II, the full field operation, will occur from October 1, 1992 to June 30, 1993; analyses will be conducted through July 1993 through June 1994.

Cancer registry staff abstract information on incident cancers in the hospitals in their respective SEER areas. The large scale population-based SEER data system covers about 10 percent of the U.S. population and, in addition to the geographic sites previously mentioned, includes the states of Connecticut and Hawaii and the reporting regions of Detroit and Atlanta. Principal investigators are responsible for consolidating all data concerning an individual cancer case and quality control.

Seven cancers have been designated for the NIA/NCI SEER Collaborative Study. They are colon, stomach, urinary bladder, prostate, breast, ovary, and cervix uteri tumors. The knowledge base on cancer in the aged will be expanded by augmenting the NCI SEER data collection in two areas: (1) documentation of the existing comorbid conditions and/or functional limitations in older persons at initial diagnosis of cancer; and (2) the illness behavior of older persons when first confronted with the signs and symptoms of cancer. The research questions are:

Do older-aged persons receive the same type of initial treatment procedures as younger persons for the same cancer sites (controlling for disease stage)? If not, to what extent are concomitant disease and/or illnesses and the normal processes of aging associated with cancer treatment in older-aged persons?

Are older-aged persons more likely to present with advanced stages of cancer at initial diagnosis? If so, what factors increase the length of time from recognition of symptoms to the time of diagnosis so that they are more likely to be seen in later stage disease?

A review will be made of diagnostic characteristics and therapeutic care of a patient sample newly diagnosed with the aforementioned tumors. Interviews of a subset of elderly cancer patients to character illness behavior will be conducted in mid-year 1993 after the full study gets underway.

2. NIA/NCI/FIC Central and East European Initiative (CEEI)

A National Institute on Aging (NIA) and National Cancer Institute (NCI) Study Team examined the potential for conducting comparative international studies on cancer in the aged with Trieste and Milan, Italy; Bratislava, Slovakia, Czechoslovakia; and Krakow and Warsaw, Poland. The NIA/NCI Study Team received support from the National Institutes of Health (NIH) Fogarty International Center (FIC) Central and Eastern European Initiative (CEEI) and their Institutes to compare common themes and concerns on cancer in persons aged 65 years and older.

The focus for each tumor registry site visit was on the data that are available from these population-based tumor registries to compare with the population-based data collected by the NCI Surveillance, Epidemiology, and End Results (SEER) Program. The exploratory meetings took place from September 28 to October 13, 1991.

The general purpose of the visits was to foster scientific collaboration in the areas of mutual interest on aging and cancer relevant to geriatrics, gerontology, and oncology and to establish a regular exchange of scientific information and encourage the exchange of scientists between the U.S. and each of the countries. Five registries were visited -- two in Italy (in Trieste and Milan); one in Czechoslovakia (in Bratislava); and two in Poland (in Krakow and Warsaw). At each tumor registry, the NIA/NCI Study Team gave a formal presentation of our data on aging and cancer and provided information on the NIA/NCI collaborative efforts underway.

Cancer affects persons age 65 years or older much more frequently than other age groups -- 55 percent of all cancers occur in this age group; 67 percent of all cancer deaths are in this age segment of the population. In the U.S. the major tumors affecting older-aged persons are breast, prostate, colon, rectum, lung, urinary bladder, pancreas, and stomach. Only recently have these facts become recognized. Therefore, the management of cancer in older-aged patients has not been studied to any large extent.

There was an initial concentration on the two research questions that have guided work in the U.S and are applicable for contrasts with the tumor registry data in Italy, Czechoslovakia, and Poland. Differences and similarities in the incidence and mortality rates of cancers between the U.S. and the selected nations offer insights on the high risk of acquiring cancer associated with advancing age, a phenomenon observed in the U.S. consistent with the data in Italy, Czechoslovakia, and Poland. Comparative international studies can provide many useful answers to questions of etiology, early detection, treatment, and the influence of aging on cancer patient management. Intercountry differences in incidence and survival for specific tumor sites stimulate development of hypotheses relevant to the association of advancing age as a high risk factor for cancer or the interrelationship of age and other risk factors.

The Fogarty International Center has been a catalyst for the NIA and NCI with the stimulus of the CEEI. Participants in the CEEI are NIA staff, Drs. Rosemary Yancik and Richard Havlik, and NCI staff, Dr. Brenda K. Edwards, and Ms. Lynn A. Ries.

3. Cancer and Aging in the Iowa EPESE

The cancer experience of Iowa EPESE respondents is being investigated by Dr. Rosemary Yancik and Dr. Richard Havlik, NIA, and Dr. Jon Lemke, University of Iowa. The Iowa data from the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program, a population-based national tumor registry, have been merged with data from the Iowa EPESE study participants. Development of the Iowa EPESE/SEER database was completed in June 1992. The SEER data augment the subgroup of 737 EPESE participants that had a diagnosis of cancer with the following information: (1) extent of disease at initial diagnosis (e.g., stage of the malignancy); (2) natural history of the tumor; (3) initial treatment; and (4) details on survival over time.

Basic descriptive questions will be asked of the merged data set, e.g.: "What chronic conditions are likely to be found in older persons with cancer?" "Were these present at initial diagnosis of the tumor?" Cancer patient/survivors will be compared with those afflicted with other chronic diseases, e.g., "Does the functional status of EPESE participants who have had a diagnosis of cancer differ from other participants diagnosed with other chronic diseases or those without a chronic disease?"

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- Persons 65 years of age and older bear the greatest burden of cancer; 55% of all malignancies occur in this age group. Sixty-seven percent of all cancer deaths occurred in this population in 1988. The magnitude of the cancer problem of the elderly is described according to major cancers (colon, rectum, lung/ bronchus, pancreas, stomach, urinary bladder, breast, and prostate.) The annual age-specific cancer incidence rate per 100,000 for persons 45-49 years of age is approximately 300. For those 10 years older, the incidence rate observed is more than double, or approximately 750. Rates increase to more than 2200 for those 75 to 79 years of age, and reach a peak of 2500 in the group 80 to 84 years of age. The major tumors affecting older women and men were contrasted. All the prominent cancers affect the elderly disproportionately. As the population of the United States ages, the cancer burden is likely to become even greater because of the increasing numbers of older persons. Researchers and clinicians working in the areas of aging and cancer are confronting the many challenges and complexities related to the interrelationship between problems of old age and cancer; the intensity of health-care resources used by older persons, the lack of information specific to the natural history of tumors in the aged, and the effects of prevention and treatment practices on the older person with cancer site-specific data. (Yancik, Ries. Cancer, 1991;68(11)2502-10.)

Epidemiology and Demography Office

The Epidemiology and Demography Office plans and conducts studies and analyses of data in the areas of environmental, social, and behavioral sciences, and interrelationships with chronic diseases and aging processes.

Staff members work under the leadership of the Office Chief, Dr. Jack Guralnik, with the Associate Director, other EDB Program staff members, other members of the NIA, other investigators at the NIH, Government contractors, the National Center for Health Statistics (NCHS), and other agencies on a variety of analytical, developmental, methodological, and administrative projects.

A great deal of work this year went into the planning and development phase of the Women's Health and Aging Study, which will enter the field in October 1992. Analytic achievements of the Epidemiology and Demography Office involved new and continuing research using data from established EDB Program projects, and collaboration with visiting scientists and researchers from outside NIH.

1. Established Populations for Epidemiologic Studies of the Elderly (EPESE)

The three original community populations comprising the Established Populations for Epidemiologic Studies of the Elderly (EPESE) are located in East Boston, Massachusetts; Iowa and Washington Counties, Iowa; and New Haven, Connecticut. Baseline data collection began in December 1981, and annual interviews were conducted either in-person (third and sixth years of follow-up) or by telephone (first, second, fourth and fifth years of follow-up) for 6 years following the baseline. A 5-year extension contract was awarded to each of the three original EPESE cohorts in February 1989. The extensions place major emphasis on data clean-up and to continued monitoring of mortality through the National Death Index and to monitoring hospital utilization through linkage to Medicare records. In the past year, links to Medicare records have been successfully completed and the quality and utility of the information obtained appears quite good. Analyses have begun that use these linked records to document hospitalization for specific events and costs of hospitalization.

Dr. Guralnik continues as Project Officer for the Iowa 65+ Rural Health Study at the University of Iowa. Pre-survey activities on a "10th-year" anniversary in person interview survey have commenced upon award of grant funding from the Behavioral and Social Research (BSR) Program, NIA. The questionnaire is being developed and pilot pretests were conducted in July. Interviewer training is scheduled for late summer or early fall, and data collection shortly thereafter.

The Piedmont Health Survey of the Elderly (PHSE) was added as the fourth site of the EPESE project in 1985. In this fourth site, Duke University has established a sample of 4,164 people 65 years old and older, 54 percent of whom are black. This cohort is the only southern EPESE population, constitutes a representative sample of the elderly in five North Carolina counties, and includes both urban and rural participants. The baseline survey for

this site began in January of 1986 and was completed in June 1987. The first and second telephone follow-ups of the sample began in January 1987 and 1988. The first telephone follow-up was completed in June 1988 and the second in June 1989. A second in-person follow-up began in January of 1989 and was completed in June 1990. The third telephone follow-up began in January of 1990 and concluded in June of 1991 with a 99.2 percent response rate. The fourth telephone follow-up began in January of 1991 and was concluded in June 1992.

A new 7-year contract was awarded to Duke University Medical Center January 1, 1991, to conduct a third in-person survey wave and continue surveillance for major endpoints using the National Death Index and Health Care Financing Administration (HCFA) hospitalization data tapes after direct contact with subjects is completed. The third in-person follow-up began in 1992 and is still in the field. Duke is gathering information comparable to that obtained by the other sites, including physical performance measures and blood samples, and also will include items which are important for the study of the health of older black persons and racial and urban/rural differences. The physical performance measures include tests of balance, a timed walk, chair stands, a test of shoulder range of motion, and functional reach. Waist/hip ratio, height and weight, skinfold thickness and arm circumference, and vision are also being ascertained. Blood assays include complete blood count, automated serum chemistries, and HDL cholesterol. A supplement to the 1991 contract was added in fiscal year 1992 to obtain additional assays of blood samples for markers of immune function, including interleukin 6, (fibrin D-dimers) and Agarose Gel Electrophoresis (AGE). Items repeated in the previous two waves which have significance for epidemiologic exploration and explaining black/white differences will also be included, since observations at three points in time will permit the kind of causal modeling for which the EPESE data are ideally suited.

Epidemiology Office staff were involved in the following EPESE data analysis activities:

Drs. Marcel Salive and Jack Guralnik have made a contribution to resolving the issue of whether left-handed individuals have a higher mortality than right-handers. Their work demonstrates that an earlier report of a lower mean age at death in left-handers implies nothing about their risk of death and probably was an artifact of the study methods. An analysis of the East Boston EPESE data demonstrated no difference in mortality by handedness. These results have been submitted for publication.

Drs. Marcel Salive and Karen Collins have recently completed a collaborative analysis, with Dr. Linda George of Duke University and Dan Foley of the Biometry Office, of the predictors of nursing home admission in the North Carolina EPESE population. Age-adjusted 3-year incidence rates of nursing home admission were 8.5 percent for whites and 6.4 percent for African-Americans. The racial differences in nursing home utilization were not explained by disability, cognitive impairment or measures of financial access and social support. These results have been submitted for presentation at the Gerontological Society of America 1992 annual meeting as well as for publication.

Drs. Salive and Richard Havlik led a collaborative analysis which examined the role of functional and cognitive limitations in the risk of pneumonia mortality in the EPESE populations. Gender-specific Cox proportional-hazards regression models were used to examine the association of baseline physical and cognitive functioning with report of pneumonia as an underlying, immediate or contributing cause of death. Adjusting for age, evidence of five chronic diseases, and smoking status, a significantly increased risk of pneumonia mortality was found for limitations in activities of daily living, inability to walk a half mile, climb stairs or perform heavy housework, and cognitive impairment in both men and women. These results were presented at the annual meeting of the American College of Preventive Medicine, Prevention '92, in March 1992.

Drs. Salive and Guralnik were instrumental in evaluating the association of sociodemographic factors with functional blindness and visual impairment in the EPESE populations. Screening for bilateral functional near and distant vision during an in-home interview in 1988 was completed by 5,335 participants. The prevalence of functional blindness increased with age, from 1 percent at age 71 to 74 years to 17 percent in those ≥ 90 years. Functional visual impairment increased from 7 percent at age 71 to 74 years to 39 percent in those ≥ 90 years. Age and nursing home residence were significantly more strongly associated with blindness than they were with visual impairment. These results have been accepted for presentation at the American Public Health Association Annual Meeting in November and for publication in the journal Ophthalmology.

Drs. Salive and Havlik performed an analysis which examined risk factors for septicemia mortality in the EPESE populations. During 6 years of followup, 177 persons (3.2 per 1,000 person-years) had septicemia reported on their death certificate. In a multivariate proportional-hazards model, septicemia mortality was significantly and independently associated with older age, male sex, history of diabetes, history of cancer requiring hospitalization, baseline smoking >one pack of cigarettes/day, not drinking alcohol, disability in activities of daily living, and cognitive impairment. Further investigation is needed to explain underlying mechanisms of increased risk of subsequent septicemia. These results will be presented as a poster at the NIH Research Festival, September 22, 1992.

Dr. Eleanor Simonsick, Mary Lafferty and Caroline Phillips completed and submitted for publication a paper entitled, "Risk due to inactivity in physically capable older adults." Several studies demonstrate the benefit of habitual physical activity, both work related and recreational, to improved health, reduced incidence of coronary heart disease, and increased longevity in middle-aged men and women. Yet, it remains unknown whether inactivity not due to disease processes increases risk of morbidity and mortality in older adults. Using data from East Boston, New Haven and Iowa EPESE sites, the authors examined the association between recreational physical activity among physically capable older adults and change in functional status, incidence of selected chronic diseases and mortality over 3 and 6 years. A high level of recreational physical activity was associated with a reduced likelihood of mortality over both 3 and 6 years. Moderate to high activity reduced the risk of physical impairments over 3 years; this effect diminished after 6 years due to differential mortality across activity groups. A consistent relationship between activity and incidence of myocardial infarction (MI), stroke, hypertension, diabetes or angina was not found after 3

years, although a protective effect of activity for MI was observed after 6 years. Findings suggest that physical activity offers benefits to physically capable older adults, primarily in reducing risk of functional decline and mortality. This paper was presented at the 1991 annual meeting of the American Public Health Association.

Dr. Simonsick has initiated a study of successful aging using data from the East Boston, New Haven, and Iowa EPESE sites. Using a pooled sample from the sixth followup, this study identifies persons age 75 to 84 years at the sixth followup who have no reported physical disability and compares them to persons alive, but disabled at the sixth followup who were free of reported disability at baseline. The intent of this analysis is to identify social and behavioral factors associated with health and longevity.

Dr. Simonsick has finalized analyses examining depressive symptomatology and hypertension associated morbidity and mortality in older adults. This paper uses data from the East Boston, Iowa, and New Haven EPESE sites. The study population consists of persons who have been told they have high blood pressure and at baseline have measured systolic blood pressure above 160mm Hg or diastolic blood pressure above 95mm Hg or take or take medication for their blood pressure. Depressive symptomatology is measured with the original or a modified version of the Center for Epidemiologic Studies-Depression (CES-D) scale. Scores from the modified forms were transformed to determine cutpoints analogous to a score of 16 on the original scale. Analyses are stratified by sex and site and compare persons with high to those with low depressive symptomatology. Hypertensive females with high depressive symptomatology show significantly greater all cause and cardiovascular related mortality after 6 years. Depressed males and females show 2- to 3-fold higher rates of stroke after 3 years. As depression is a condition that often responds well to treatment, recognizing and understanding its role in the development of more severe disease is highly desirable. A version of this paper was presented at the Sixth National Conference on Chronic Disease Prevention and Control sponsored by the Centers for Disease Control, October 1991.

Drs. Simonsick and Guralnik are completing analyses of the prevalence of symptomatic intermittent claudication (IC) and associated conditions and risk factors. These analyses also examine mortality and occurrence of MI stroke and disability in activities of daily living over a 6-year period in claudicants with and without other manifest cardiovascular conditions relative to non-claudicants. Persons with symptomatic IC are sicker than persons with no IC, in that they report significantly higher rates of angina, past MI, diabetes, hypertension and respiratory symptoms, particularly shortness of breath and moderate disability. Symptomatic IC is highly predictive of poor outcome over both a short- and longer-term followup, even when differential comorbidity is taken into account. This paper has been accepted for presentation at the 1992 annual meeting of the American Public Health Association.

Dr. Simonsick is performing analyses that examine the demographic, health and functional characteristics of persons age 65 to 69 years who are still working in comparison to persons in the same age group who are no longer working, including and excluding persons who stopped working due to disability. The analysis follows this population of older workers for 5 years to prospectively evaluate factors associated with retirement and continued working,

primarily from a health status and health event perspective. The major hypothesis is that persons who are still working at age 65 years and beyond will continue to work until they no longer have the physical or mental capacity to do so. This will be related to occupational type as different occupations have different physical and cognitive demands. Social factors such as the loss of a spouse may also be related to continued working. The analyses are done separately for men and women within each EPESE site. While many studies examine predictors of retirement, few examine factors associated with continued working among persons of retirement age.

Drs. Jack Guralnik and Andrea LaCroix have completed work on a longitudinal study of factors that predict the preservation of high levels of functioning in older persons. Factors predicting maintenance of mobility were studied in 10,296 persons aged 65 and older residing in three communities of the EPESE (East Boston, Iowa, and New Haven). Companion papers from these analyses have been submitted together for publication. The first addresses the demographic characteristics and chronic conditions that predict the maintenance of mobility, defined as the ability to climb stairs and walk a half mile without the help of another person. In both men and women, increasing age and lower income levels were associated with increased risk of losing mobility, even after controlling for the presence of chronic conditions at baseline. Lower education levels were a significant risk factor for mobility loss in men but not in women. Baseline reports of previous heart attack, stroke, high blood pressure, diabetes, dyspnea, and exertional leg pain were associated with small but significant risks for mobility loss. There was a stepwise increase in the risk of mobility loss according to the number of chronic conditions present at baseline that was very consistent between men and women. The occurrence during the study of a new heart attack, stroke, cancer, and hip fracture was associated with a substantially greater risk of mobility loss than that associated with the presence of these conditions at baseline.

The second paper addresses the association of health behaviors and maintenance of mobility. After adjustment for age and all health behaviors, risk of losing mobility was significantly associated with current smoking, not consuming alcohol compared to small to moderate amounts of alcohol consumption, high (> 80th percentile) as compared to moderate (21-80th percentile) body mass index, and high physical activity levels in both men and women. These findings suggest that positive health behaviors can not only extend longevity but reduce the risk of losing mobility and independence in later life.

In a supplemental study to research completed on factors predicting maintenance of mobility, Dr. Guralnik has been investigating the role of depression in the pathway from disease to disability. Symptoms of depression were assessed using modifications of the CES-D scale. Adjusting for age and eight chronic conditions found to be associated with loss of mobility, depression was found to be significantly associated with loss of mobility in both men and women. The independent association continued to be statistically significant after adjusting for the burden of disease, represented as the total number of chronic conditions, and for conditions newly occurring during the follow-up period. These results were presented at the annual meeting of the Gerontologic Society of America, November 1991 and at the 50th Annual Meeting of the American Psychosomatic Society, April 1992, and will be submitted for publication.

Drs. Guralnik, Simonsick, and Luigi Ferrucci continued analyses of the objective measures of physical performance collected in East Boston, Iowa, and New Haven in the 1988 assessment. Analyses have assessed the association of three lower extremity performance measures done in over 5,000 individuals and self-reported disability. The performance measures include a timed walk, a timed test in which the individual is asked to stand up and sit down five times as quickly as possible, and a test of balance using side-by-side, semi-tandem, and tandem stands. Self-reported measures include activities of daily living needing lower extremity function (walking across a room, bathing, transferring, and using the toilet) and higher level functions (climbing stairs and walking a half mile). Each performance test and a summary measure of the three tests were strongly associated with self-report of disability. The self-report items predicted 47 percent of the variance in the summary performance test score. However, for each level of function as defined by the self-report, there was a wide distribution of scores in the performance tests. The initial impression of these results is that self-report and performance measures complement each other. Dr. Guralnik will present the results of these analyses at the World Health Organization workshop "Application of Physical Performance Measures in International Studies" in September 1992 and at the Gerontologic Society of America meeting in November 1992.

Dr. Guralnik has completed an investigation into active life expectancy using data from the Duke EPESE. This paper, entitled, "The Impact of Educational Status on Life Expectancy and Active Life Expectancy in Older Blacks and Whites," will soon be submitted for publication. Black men age 65 years had lower life expectancy (LE) and active life expectancy (ALE) than white men, although this differential was reduced after stratification for education. Black women age 65 years were similar to white women. Black men and women age 75 years and older had higher LE and ALE than whites and these differences were accentuated after stratification for education. Education had a substantially greater impact on LE and ALE than race. At age 65, higher educated persons (12 years of education or greater) had an ALE 2.4 to 3.9 years greater than those with lower education in all sex/race subgroups. Overall, those subgroups who had longer LE and ALE also lived more years in the disabled state. Dr. Guralnik will present the results of this investigation at the American Public Health Association Meeting in November 1992.

Drs. Lori Brown and Guralnik are working on an analysis to determine whether estrogen use by women at the time of menopause protects against hip fracture later in life. There is evidence that estrogen replacement therapy (ERT) at the time of menopause prevents bone mass loss but it is not known whether delaying bone loss at the time of menopause may have continued protective effects against osteoporotic fractures as women age. Another factor may be the length of ERT, that is, longer therapy may influence later hip or bone fracture whereas short term therapy for the symptoms of menopause may have little effect.

Drs. Brown, Guralnik and Salive have begun a study to determine whether depression may affect immune cells as measured by differences in white blood cell counts in depressed elders from their undepressed counterparts. The depressive symptomatology was measured with a modified CES-D instrument. Those who exhibit high depressive symptomatology will be

compared to the remainder of the cohort with respect to their levels of total and specific white blood cells. Dr. Brown has an extensive background in investigating immune neuroendocrine interactions.

Dr. Brown, Dan Foley, and Dr. Marco Pahor are collaborating with Dr. Elizabeth Chrischilles of the University of Iowa in a study to investigate the characteristics of elders who use no medications. In the EPESE, preliminary data indicates that among non-drug users, there is a subpopulation whose self reported health status is fair to poor but who use neither prescription nor over the counter drugs. A study of the characteristics of this group with respect to disease status, use of medical services, disability, cognitive function, depression, socioeconomic status and mortality are planned.

Drs. Brown and Harris are planning an analysis of the influence of weight loss on risk of hip fracture. It is known that older persons in poor health may lose weight. Because lower weight is associated with decreased bone mass, weight loss may be a factor in hip fracture. Recent studies have indicated that Interleukin 6 (IL-6), a factor produced by cells of the immune system in response to inflammatory stimulus or cancer, may cause a decrease in bone mass by stimulating osteoclast. Because IL-6 may also have cachectic activity, this may be the link between ill health, weight loss, decreased bone mass and subsequent fracture.

Dr. Heidi Jolson, a visiting researcher from the Food and Drug Administration, and Dr. Guralnik are completing a study entitled, "Socioeconomic and Demographic Determinants of Hypertension Therapy Status in Older Persons." This work will shortly be submitted for publication.

Drs. Luigi Ferrucci and Sergio Costanza, visiting scientists from Florence, Italy, and Dr. Guralnik analyzed data from the Iowa site of the EPESE to investigate the association of orthostatic hypotension, assessed in the sixth annual followup in 1988, with time of day. The hypothesis for this study is that orthostatic hypotension is more common after meals.

Drs. Pahor and Guralnik have begun analyses of data from the Iowa EPESE study on the use of laxative medication in the older population. Most are over the counter drugs and little is known on the safety of these self-prescribed drugs. Frail elderly with limited physical activity often suffer constipation, are high consumers of laxatives, and are at risk for electrolyte imbalance and dehydration. Furthermore, a chronic drug-induced diarrhea can facilitate malabsorption and malnutrition. On the other hand, some studies have reported beneficial hypocholesterolemic effects of different bulk-forming hydrophilic fibers. Thus, a better understanding of the impact of specific laxative drugs on bowel movement habits, on nutritional status, on serum sodium, potassium, calcium and cholesterol levels, and on interactions with other frequently prescribed drugs such as diuretics would help in understanding the risk/benefit ratio of such medication.

Drs. Pahor, Guralnik, and Salive have begun research on the incidence and risk factors for gastrointestinal hemorrhage in the elderly, using data from the Iowa EPESE interviews and newly available Medicare hospitalization records obtained by linking the Iowa cohort to

HCFA records. Gastrointestinal hemorrhage (GIH) is a frequent cause of hospital admission in the elderly and has been shown to increase with aging. Major events have a fatality rate of 15 percent. The episodes are mainly correlated with nonsteroidal anti-inflammatory drugs or anticoagulant therapy. Research using the EPESE data will examine how specific conditions often related to aging, such as disability, cognitive impairment, decline in physical activity and poor compliance to drug prescriptions may be independently associated with GIH, and may explain the increased rate of GIH with age. Identification of a high risk and a low risk elderly population will be useful for risk/benefit evaluation in making decisions on drug prescription.

2. Women's Health and Aging Study (WHAS)

On July 1, 1991, the Johns Hopkins University School of Medicine was contracted to perform the study over the period from July 1, 1991, through February 28, 1998. Data collection will be accomplished through a subcontract with Westat, Inc. Application for OMB clearance of the research was submitted in March 1992 and final clearance was obtained in July 1992. In October 1992 field staff will begin screening the first of about 5,500 older women in Baltimore County and City for eligibility into the study.

A number of important hypotheses related to disability and loss of independence in older women will be addressed by this study. The project will enroll 1,000 disabled women in the study cohort, who will receive an extensive baseline evaluation and be followed prospectively. At baseline, data will be collected on the major diseases and conditions of aging to ascertain their presence, severity, and impact on disability. The cohort will be followed for 3 years, with in-home follow-up assessments every 6 months to evaluate change in disease and functional status. Members of the cohort will also be monitored for hospitalization, nursing home admission, the use of home health care, and mortality. Dr. Guralnik is the Project Officer for the Women's Health and Aging Study.

This study has a number of aspects that make it unique as an epidemiologic study and add substantially to previous work done on disability in older populations. Epidemiologic studies in general have tended to study factors leading to the onset of incident disease, with little study of subjects once disease occurs. This study will examine women who already have disease and disability, attempt to understand the diseases underlying that disability and then prospectively evaluate the course of disability and how the underlying diseases as well as health habits, psychological, cognitive, social and other factors affect that course. Unlike previous epidemiologic research on disability, this study will intensively evaluate diseases and physiologic dysfunction that are associated with disability. For example, through the use of chart reviews of previous hospitalizations, physical examinations, resting electro-cardiograms (ECG's), ambulatory ECG's, pulmonary function tests, modified exercise stress tests, and oximetry readings of blood oxygen saturation both at rest and during exertion a thorough understanding is sought of the association of cardiopulmonary disease and disability in this community-based, representative sample of disabled women. Likewise, knee and hip x-rays, photographs of the hands, and a clinical joint examination will elucidate the association of arthritis and disability in this disabled cohort. The 6-month interval between

follow-up assessments, as compared to the usual annual follow-up, should allow for a better ability to characterize change in functional status, which may fluctuate rapidly over a short period of time. A supplementary study of a subset of the participants will perform weekly assessments over a 6-month period to evaluate very short-term changes in functioning.

The first year of the study has been primarily concerned with planning, development of assessment protocols, creation of an OMB package, and pre-testing of questionnaire and examination instruments.

Two rounds of pretests were carried out during January and February 1992, to assess (1) the time required to administer the questionnaires and examination, including timings for individual subsections, and (2) to assess the acceptability of the interview and examination to participants. The volunteers were similar in age and disability status to those who will be eligible for the study. These individuals participated in the screener, the baseline interview, and the nurse practitioner examination. Based on these pretests, instruments were modified and finalized prior to submission for OMB clearance in March 1992.

The clinical investigators on the study team have developed algorithms specifying criteria for determining both presence and severity of disease. The algorithms take into account information obtained by respondent report (from the interviews), by clinical assessment (from the home examination), and from medical records (through the surveillance activity). The algorithms cover the following diseases and conditions: cancer, angina, coronary heart disease, congestive heart failure, status post myocardial infarction, intermittent claudication, fractures, osteoarthritis of hands, hips, and knees, rheumatoid arthritis, disc disease, stroke, peripheral neuropathy, Parkinson's Disease, pulmonary disease, diabetes mellitus, and vision impairment.

The process of developing and revising the algorithms is nearly complete. Work has begun on operationalizing them with regard to the specific data elements required, from what sources they will come, and how they will be incorporated into the data management system and compiled for use in the adjudication process, in which clinician investigators will make judgements on the presence and severity of diseases and the diseases directly responsible for the disability observed in study participants.

The HCFA Medicare enrollment files, the sampling frame for this study, have been received. The total number of women aged 65 and over in the geographic area is 32,539. Participants in the Current Beneficiary Study (N=16) and in the Senior Health Watch project (N=2,258) were removed, leaving a sampling frame of 30,266, a number very close to that estimated in the proposal. The age and race distributions are also similar to those estimated earlier, though there is a somewhat higher than expected proportion of black women (21 percent as compared to the estimate of 16 percent). The samples for the first replicate were drawn in mid-July.

The current schedule for the project calls for the screening and baseline interviewing to begin October 1992. Development of procedures and materials for training is underway. The nurse practitioner training will begin September 1, and interviewer training will be conducted

September 14 to 22. Equipment and supplies are being ordered, and arrangements have been made to have fitted cases made for equipment used in the nurse practitioner examination. Detailed specifications on all interview questions and examination components will be completed prior to the beginning of training.

A great deal of work has been done to present the study to the community with the aim of informing potential respondents, their families and health care providers about the study, and increasing the rates of participation in the study in its initial phases and retention over the long term. Endorsements have been received from the Area Agency on Aging, the Maryland State Office of Aging, a local committee of clergy, and the President of the Baltimore City Council. The Area Agency on Aging, which operates a series of nutrition programs, including "Eating Together" and Meals-On-Wheels, will help distribute a flyer describing the study in very general terms. Approval has been obtained from the state medical society, the Medical and Chirurgical Society of Maryland, to mail a descriptive summary of the project to physicians in the Central Maryland area. Further activities will be planned in collaboration with the Johns Hopkins University's public relations department. Plans for posters and flyers are being developed, and consideration is being given to public service announcements in the media.

The contract for the Women's Health and Aging Study has been modified to specify the use of computer-assisted personal interviewing (CAPI) in the baseline and follow-up interviews. Specifically, this entails supplying each interviewer with a notebook computer which is used to enter responses to questions directly. While there are additional costs associated with this process, related primarily to programming and equipment leasing, there will be improvements in the quality of the information obtained and in the timeliness with which the data will be available for analysis. In particular, CAPI will facilitate the linkage of information between interviewing waves, so that appropriate follow-up questions can be asked based on information obtained in previous interviews.

The NIA Project Officers for this study are Drs. Jack Guralnik and Eleanor Simonsick.

3. Head Injury and Alzheimer's Disease

Dr. Salive has completed a pilot study of the feasibility of performing a study of head trauma as a risk factor for Alzheimer's disease. In collaboration with the Institute of Medicine Medical Follow-up Agency (MFUA), a sample of 300 persons was drawn from the World War II Battle Casualty Registry, their records were obtained and reviewed for documentation of head injury and other details. On the basis of the pilot study results, a more definitive study is being developed.

4. Analytic and developmental accomplishments by Epidemiology Office staff.

Drs. Ferrucci and Guralnik completed research and submitted a paper on the impact that overall health status and common chronic medical conditions have on cognitive performance in older persons who do not have dementia, and the effect that poor cognitive performance has on functional disability in these individuals. After adjusting for educational level,

physical health status and several specific chronic conditions were predictors of cognitive performance. A significant association was found between lower cognitive performance and poorer functional status. In a logistic model, better cognitive performance was associated with a reduced risk of moderate and severe disability, independent of the effects of education. Results suggest that the cognitive impairment secondary to poor health status can be documented, and is, at least in part, involved in the pathway from disease to disability. This research was presented at the annual meeting of the Gerontologic Society of America, November 1991.

Drs. Ferrucci and Guralnik completed research and submitted a paper on recovery of functional status after stroke. It is a study that follows disability and functional limitations in stroke patients after they leave rehabilitation. This research documented continued functional recovery during the 6-month follow-up after discharge from rehabilitation. Improvement in disability over time was limited to those who entered the study with a greater degree of neuromuscular impairment. Within this group, those age ≥ 65 years had more disability at baseline than younger individuals but had the same slope of improvement as those < 65 years old. However age was found to modify the relationship between neuromuscular impairment and disability. In those < 65 years old change in disability over time was completely explained by change in neuromuscular function. This was not the case in those ≥ 65 years, in whom functional recovery was greater than that expected from their improvement in neuromuscular impairment alone. These results provide evidence that improvement in disability in older stroke patients may result from the employment of "compensatory strategies" that overcome some of the neuromuscular impairment which remains following stroke.

Dr. Guralnik serves on the Editorial Boards of the Journal of Aging and Health and the journal Revista de Gerontologia, published in Barcelona, Spain.

Dr. Guralnik was the Course Director for both the Introductory and Advanced Geriatric Epidemiology Courses in Padua, Italy. These courses were taught to geriatricians who are beginning to work in clinical and epidemiologic research and provided basic instruction in epidemiologic techniques, approaches to epidemiologic research in aging, and opportunities for students to consult on their own research.

Dr. Guralnik spoke on the active life expectancy and the compression of morbidity in the course The Epidemiology of Aging at the Johns Hopkins University School of Hygiene and Public Health.

Dr. Guralnik is consultant to a project on the financial and medical impact of rationing high technology health care in the older population, Principal Investigator Dr. Edward Schneider, Dean, Andrus School of Gerontology, University of Southern California. Project funded by the Retirement Research Foundation.

Dr. Guralnik is consultant to the Human Population Laboratory Alameda County Study research project in which the older members of this cohort were re-surveyed in 1989. The Principal Investigator is Dr. George Kaplan, Director of the Human Population Laboratory

and the survey and analysis of data is funded by the Kaiser Family Foundation. Dr. Guralnik travelled to Berkeley to work with the staff doing analyses for this project in October 1991.

Dr. Guralnik consulted with the World Health Organization Project on Determinants of Healthy Aging. He will participate in their workshop on "Application of Physical Performance Measures in International Studies" in September 1992.

Dr. Guralnik consulted with Dr. Stefania Maggi on her community research project in the Veneto region of Italy, "The Quality of Life in the Elderly in Veneto." Drs. Guralnik and Maggi are currently analyzing data on physical performance measures in the Veneto and EPESE studies to compare levels of functioning and how self-reported disability relates to objective performance in the U.S. and Italy. Results of this research will be presented at the Gerontologic Society of America meeting in November 1992.

Dr. Guralnik has consulted extensively with Dr. Dorly Deeg of Rotterdam, The Netherlands, on a national study on the health of the elderly for which she is the principal investigator. A number of aspects of this study will be comparable to the EPESE studies as a result of these consultations.

Dr. Guralnik has consulted with INRCA, the Italian National Research Institute on Aging Care, on the follow-up of the European Eleven-Country Study of the Elderly. In October 1992, he will attend a meeting in Florence of investigators from several of the original countries involved in the study as an observer and discussant. The purpose of this meeting is to plan a 10-year follow-up of the original studies.

Dr. Guralnik has offered continuing consultation to researchers at the Centro Universitario de Salud Publica in Madrid and the Spanish National Institute of Health (FISS), which are planning a multicenter epidemiologic study of aging that will be initiated in 1993. He will travel to Madrid in December 1992 to speak on EDB Program research and to provide further consultation on their study.

Dr. Guralnik consulted with a number of groups and researchers in the U.S., including: Alliance for Aging Research, Washington, D.C.; Dr. Robert Epstein, Director of Epidemiologic Research, Merck, Sharp, and Dohme; Dr. Regula Herzog, University of Michigan, Institute for Social Research; Dr. David Rabin, Department of Community and Family Medicine, Georgetown University School of Medicine; Dr. Jay Magaziner, Department of Epidemiology and Preventive Medicine, University of Maryland Medical School.

Dr. Guralnik serves as EDB Program representative to the MacArthur Project on Successful Aging. He serves on the Steering Committee to monitor research and publications and was designated to lead the research efforts on the performance measures of functioning used in the MacArthur study. Members of the EPESE cohorts with no self-reported disability and good cognitive functioning were selected to participate in the MacArthur supplementary study on successful aging. Research is under way to evaluate whether performance measures of

functioning can serve to accurately represent the hierarchy of functioning in these individuals at the high end of the functional spectrum. Analyses thus far show distributions of times for the performance measures that are associated in the expected ways with demographic characteristics and chronic conditions. When follow-up data are available, the ability of the baseline performance measures to predict adverse outcomes such as functional decline, nursing home admission, and death will be evaluated. Results from this research were presented at the Gerontologic Society of America meeting in November 1991 and will be submitted for publication.

Drs. Pahor and Guralnik recently completed research and submitted a paper on the association of age and other potential risk factors with adverse drug reactions to digoxin. This was a multicenter survey done in 22 internal medicine and 19 geriatric wards throughout Italy. The analyses used all patients (n=1,338) consecutively admitted during two observation periods of 2 months in 1988 who were on digoxin therapy at the time of admission.

Drs. Salive and Havlik have provided ongoing consultation to the Equal Employment Opportunity Commission in their work on characterizing the physical and cognitive functioning of older pilots.

Dr. Salive served as institute co-representative to the NIH working group on the Health Care Financing Administration (HCFA) Medicare Beneficiary Registry.

Dr. Salive served as institute representative to the workgroup on professional education of the PHS Task Force on Improving Medical Criteria for Disability Determination.

Dr. Salive was designated as the USPHS Delegate to the AMA Young Physicians Section by the Surgeon General for a term of 1991 to 1993; he provided representation to the annual and interim meetings 1991 to 1992.

Dr. Salive served as a manuscript reviewer for the following professional journals: Health Services Research; Military Medicine; American Journal of Public Health; American Journal of Preventive Medicine; and Tobacco Control--an international journal. He also reviewed a dissertation grant proposal for the Agency for Health Care Policy and Research.

Dr. Simonsick, in collaboration with Dr. Jay Magaziner from the University of Maryland School of Medicine and Dr. T. Michael Kashner from the University of Arkansas School of Medical Sciences, completed an analysis examining the circumstances surrounding fall related hip fractures in community dwelling elderly and relationships between selected patient characteristics and attributes of the fall. A majority of the fall related hip fractures in community dwelling persons age 65 years and older occurred in healthy individuals who fell in the course of performing routine activities. Psychotropic drug use was not excessive and the prevalence of physical limitation was low. Patients who had a previous hip fracture and persons who had concurrent neurological disturbances were more likely to fracture in the nocturnal hours and in late afternoon and early evening hours, respectively. In order to prevent hip fracture in older adults without limiting usual activities, preventive efforts need

be directed at increasing activity to improve fitness and thereby reduce the risk of falling. The paper entitled, "Fall related hip fractures in community dwelling older adults: Are they preventable?" has been submitted for publication.

Dr. Simonsick completed and submitted for publication,"Relationship between Husband's Health Status and the Mental Health of Older Women". This study examines the association between husband's health and the mental health of community dwelling white women 65 to 75 years of age and how the wife's health, socioeconomic and social resources and marital quality affect this relationship. Husband's health strongly predicts wife's mental health; the negative impact of which is more pronounced when the wife has poor or declining health as well. Marital quality is the strongest predictor of the wives' mental health. Analyses examining the independent and joint effects of marital quality and husband's health on wife's mental health indicate that the negative association of illness in a spouse on wife's mental state is, in part, a function of the impact husband's health has on marital intimacy and shared pursuits. Of the socioeconomic and social resources examined, only availability of close friends shows a substantial relationship to the wife's mental health.

Dr. Simonsick has initiated a study of successful aging using data from the Longitudinal Study of Aging. This analysis begins by identifying persons age 80 to 84 at the time of the 1988 followup who have no reported physical disability and compares them to two populations: (1) persons alive, but disabled in 1988 and (2) persons deceased since the 1984 baseline survey. The comparison populations will be examined in total and after eliminating persons with disability at the time of the initial survey. The intent of this analysis is to identify social and behavioral factors associated with health and longevity and maintaining health in aging.

Dr. Simonsick serves as a consultant to the Health and Retirement Study Monitoring Committee. The Health and Retirement Study (HRS) is being conducted by the University of Michigan as a cooperative agreement with the Behavioral and Social Research Program, NIA. The objective of the HSR is to describe the economic, health and other factors that influence retirement decisions as well as to track changes in health status and economic well-being as the panel ages. It is designed as a longitudinal survey of a large national sample of individuals approaching retirement age.

Dr. Simonsick is a member of the Community Randomized Trial (CRT) working group of the Women's Health Initiative (WHI). The WHI is a broad ranging research program designed to evaluate the effects of preventive interventions on cancer, cardiovascular disease and osteoporosis in women. The CRT component of the WHI aims to identify effective ways of achieving healthful behaviors related to dietary pattern, nutritional supplementation, hormone replacement therapy, smoking cessation and prevention, physical activity and cancer detection.

Dr. Simonsick serves as a consultant to the National Cancer Institute for their study on the correlation between diet, weight, and physical activity: Longitudinal Analysis of Behaviors Related to Diet, Weight, and Physical Activity: Followup to the 1992 National Health Interview Survey Cancer Supplement.

5. Talks and Presentations

Ferrucci L, Guralnik JM, Marchionni N, Costanzo S, Lamponi M, Baldan A, Gavazzi P, Baroni A. The role of cognitive impairment in the pathway from disease to disability: evidence from an Italian cohort. Presented at the 44th Annual Scientific Meeting of the Gerontologic Society of America, San Francisco, November 1991.

Guralnik JM, Land K. The Impact of race and educational status on life expectancy and active life expectancy in a cohort of older persons. Presented at the Duke University School of Medicine. Durham, September 1991.

Guralnik JM, Seeman T, Berkman L, Tinetti M, Nevitt M: Physical performance measures in a highly functional cohort of older persons: Initial results from the MacArthur Studies of Successful Aging. Presented at the 44th Annual Scientific Meeting of the Gerontologic Society of America, San Francisco, November 1991.

Guralnik JM, LaCroix AZ, Kohout FJ, Berkman L: The role of depression in the pathway from disease to disability. Presented at the 44th Annual Scientific Meeting of the Gerontologic Society of America, San Francisco, November 1991.

Guralnik JM: Health status of older adults: Major factors in morbidity and mortality. Presented at the meeting Social Science and Health Policy: Building Bridges Between Research and Action. Bethesda, March 1992.

Guralnik JM: Epidemiologic studies of behavioral and psychological factors in aging. Presented at the 50th Annual Meeting of the American Psychosomatic Society, New York, April 1992.

Salive ME, Cornoni-Huntley J, Phillips CL, Hennekens CH, Ostfeld AM, Wallace RB. . Predictors of smoking cessation and relapse in older adults: The influence of chronic disease and alcohol consumption. Sixth National Conference on Chronic Disease Prevention and Control: Making Prevention a Reality. October 1991.

Salive ME, Cornoni-Huntley J, Phillips CL, Hennekens CH, Ostfeld AM, Wallace RB. Smoking Cessation in the Elderly: Results from EPESE. American Public Health Association, 119th Annual Meeting, Atlanta, November 1991.

Salive ME, Cornoni-Huntley, Phillips CL, Guralnik JM, Cohen HJ, Glynn R, Ostfeld AM, Wallace RB. Anemia and hematologic indices in older persons. American Public Health Association, 119th Annual Meeting, Atlanta, November 1991.

Salive ME, McClendon J. Preventive medicine physicians--earnings and practice. American Public Health Association, 119th Annual Meeting, Atlanta, November 1991.

Salive ME, Cornoni-Huntley, Guralnik JM, Phillips CL. Relationship of serum albumin and age in older persons. Gerontological Society of America, San Francisco, November 1991.

Salive ME, Ostfeld AM, Wallace RB. Pneumonia mortality over 65: Is disability a risk factor? Prevention '92, Baltimore, March 1992.

Salive ME. Evaluation of Aging Pilots: Evidence, Policy, and Future Directions. Aerospace Medical Association 63rd Annual Meeting, Miami Beach, May 1992.

Salive ME, Wallace RB, Ostfeld A, Havlik R. Smoking as a Risk Factor for Septicemia Mortality in Older Adults. NIH Research Festival 1992. September 1992.

Simonsick E, Glynn R, Blazer D, Wallace R. Symptoms of depression and hypertension-associated morbidity and mortality among older adults. Presented at the Sixth National Conference on Chronic Disease Prevention and Control: Making Prevention a Reality, Washington, DC, October 1991.

Simonsick EM, Lafferty ME, Phillips CL, de Leon CM, Kasl SV, Seeman TE, Fillenbaum G, Hebert P. Risk of inactivity in physically capable older adults. Presented at the 119th Annual Meeting of the American Public Health Association, Atlanta, November 1991.

6. New Staff

S. Lori Brown, Ph.D., M.P.H. joined the Office July 2, 1991, as an Epidemiology Staff Fellow. She was recruited for the U.S. PHS Epidemiology Training Program whose mission is to attract qualified professionals into the field of epidemiology. Dr. Brown has training in biomedical sciences, with an area of specialization in immunology. She has experience in laboratory research, experimental design, and statistical analyses. She has career plans to use epidemiologic techniques to conduct population studies in older persons which relate to immunologic function. Dr. Brown received her M.P.H. degree from The Johns Hopkins University School of Public Health in June 1992 and began work in Bethesda.

Dr. Luigi Ferrucci, M.D., Ph.D., from the Italian National Institute on Aging and the Ospedale I Fraticini in Florence was a Visiting Associate in the NIH Visiting Associate Program from October 1991 through June 1992. Dr. Ferrucci is a geriatrician with experience in clinical and epidemiologic research. During his stay he collaborated with several EDB Program scientists on research projects employing data from Italian and NIA studies.

Dr. Marco Pahor, M.D., from the Division of Gerontology of the Universita Cattolica del Sacro Cuore in Rome, will be a guest researcher from July through November 1992. Dr. Pahor is a geriatrician with extensive experience in clinical and laboratory research who has begun doing epidemiologic investigations in the past few years. His main area of interest and expertise is in pharmacoepidemiology. He will spend his time in the EDB Program analyzing data from a multicenter study of adverse drug reactions he coordinated and data on drug utilization collected in the EPESE study.

Research Highlights FY92

- In hematologic tests obtained from 3,946 adults aged $\geq$71 years in three EPESE communities (East Boston, MA; Iowa and Washington counties, IA; and New Haven, CT), it was concluded that age was significantly associated with both hemoglobin levels and anemia, with a stronger effect in men compared with women, even after simultaneously adjusting for demographic characteristics and health status. The decline of hemoglobin and concomitant increased anemia with age was not necessarily a result of "normal aging" so the detection of anemia in an older person should prompt appropriate clinical attention. Hemoglobin level was inversely associated with age, although this was more pronounced in men than in women. The proportion anemic was equal for men and women aged 71-74 years (8.6%) and increased differentially with age, reaching 41% and 21% for men and women aged $\geq$90 years, respectively. Hemoglobin and anemia were independently associated with age, race, body-mass index, smoking, cancer, hospitalization, renal insufficiency, and hypoalbuminemia. The adjusted relative odds of anemia for a 5-year increase in age was 1.5 (95% [CI] 1.3-1.8) for men and 1.2 (95% CI 1.1-1.4) for women. (Salive, Cornoni-Huntley, Guralnik, Phillips, Wallace, Ostfeld, Cohen. J Am Geriatr Soc, 1992;40:489-96.)

- Data were analyzed from 4,115 persons aged 71 years and older who had blood drawn at a home visit in three EPESE communities to examine the cross-sectional distribution of serum albumin and correlates of hypoalbuminemia. Mean albumin was lower among older persons, from 41.6 g/l in men aged 71-74 years to 38.5 g/l in men 90 years or older, and from 41.1 g/l to 38.9 g/l in women of the same ages, respectively. Hypoalbuminemia (albumin <35 g/l) was observed in 3.1% of subjects. Hypoalbuminemia and lower serum albumin were independently associated with anemia, recent diagnosis of cancer, two or more limitations in activities of daily living, residence in a nursing home, heavy cigarette smoking (>1 pack/day), and older age. A 10-year age increment was associated with 0.8 g/l lower serum albumin and odds ratio of 1.56 (95% CI 1.14, 2.13) for hypoalbuminemia after adjusting for demographic factors and health status. Characteristics associated with serum albumin may confound the reported relationship between serum albumin and mortality. (Salive, Cornoni-Huntley, Phillips, Guralnik, Cohen, Ostfeld, Wallace. J Clin Epidemiol, 1992;45:213-21.)

- Longitudinal changes in smoking behavior were examined among older adults in three community cohorts of the EPESE. Smoking prevalence declined from 15% at baseline to 9% during 6 years of follow-up. Annual smoking cessation and relapse rates were 10% and less than 1%, respectively. Interval diagnosis of myocardial infarction, stroke, or cancer increased subsequent smoking cessation but not relapse. Although smoking cessation around diagnosis is increased, primary prevention could yield greater benefits. (Salive, Cornoni-Huntley, LaCroix, Ostfeld, Wallace, Hennekens. Am J Public Health, September 1992 [in press]).

- A shortage of preventive medicine (PM) physicians exists inthe United States. Researchers know little about thesephysicians' earnings and practice characteristics. The

American College of Preventive Medicine (ACPM) mailed a survey to all self-identified PM physicians on the American Medical Association (AMA) Physician Master file. A total of 3,771(54%) responded; respondents' sex and region of residence were typical for PM physicians in general, with a slight excess of older physicians and those reporting board certification. A total of 2,664 (71%) were working full time, with median earnings of $85,000 (mean $90,000). Among full-time physicians, relatively higher earnings were associated with the following characteristics: male sex; age 45 to 64 years;major source of income from clinical, business, or industrial sources, rather than governmental or academic; and PM board certification. Full-time PM physicians earned much less than office-based private practitioners in several primary care specialties in 1989. The gap in earnings between PM specialists in government positions and those in the private sector is also substantial. Both disparities may require creative solutions. (Salive. Am J Prev Med, 1992;8:256-261).

- Little is known about those physicians who pursue graduate medical education in preventive medicine, including aerospace medicine, general preventive medicine and public health, and occupational medicine. Resident physicians were surveyed about their academic background, financial environment, clinical activities, and professional goals. A total of 147 residents (30%) responded from a population of 498 residents. The data suggest a lack of available information about preventive medicine training and careers among medical students who subsequently pursue such training. Their economic environment is extremely diverse, with a wide range of salary, "moonlighting" hours, educational loans, and service obligations. Although the median annual salary ($24,700) is similar to the national average resident salary, 32% of respondents earned less than $20,000, and 95% have educational debts averaging $30,900. Sixty-two percent of respondents perform clinical work in their residency, whereas 76% desire future clinical work as part of their practice. This gap is most pronounced in general preventive medicine and public health. The residents express a wide range of interests in future practice of preventive medicine; 54% are interested in government work, and 33% desire academic careers. (Salive, Parkinson. Am J Prev Med, 1991;7:445-9).

- Data from three EPESE populations suggest that the relationship of low to moderate alcohol consumption with reduced total and cardiovascular mortality, which are well documented in middle age, also occur in older populations. Subjects with prior myocardia infarction, stroke, or cancer, as well as those lacking alcohol consumption data, were excluded from statistical analyses leaving 2,694 subjects in East Boston, MA, 2,293 subjects in Iowa, and 1,904 subjects in New Haven, CT. Main outcome measurements were alcohol consumption, total mortality, cardiovascular mortality, and cancer mortality. Low to moderate alcohol consumption was associated with statistically significant lowered total as well as cardiovascular mortality in East Boston and New Haven. The relative risks of total mortality for low to moderate consumers of alcohol compared to those consuming no alcohol in the previous year were 0.7 (95% CI 0.6-0.8) in East Boston and 0.6 (95% CI in New Haven. For cardiovascular mortality, the RRs were 0.6 in East Boston and 0.5 in New Haven (95% CI's exclude null). These results persisted after control for potential confounding variables. In Iowa, there were no significant differences in total cardiovascular mortality according to alcohol consumption patterns. For cancer mortality, there were no

significant associations with alcohol consumption in any of the three populations. (Scherr, LaCroix, Wallace, Berkman, Curb, Cornoni-Huntley, Evans, Hennekens. J Am Geriatr Soc, 1992;40:651-57.)

- The relationship was examined between 6 of 7 Alameda County health habits and four dietary practices and 3 indices of mental health in a probability sample of 2,436 adults 20 to 64 years of age, residing in the coterminous United States. The sample consisted of all respondents to Wave 2 of the National Survey of Personal Health Practices and Consequences conducted in 1980. A strong relationship was found between poor health habits and risk of depressed mood and "nervous breakdown." The magnitude and direction of the association varied by sex, age group, the specific habit examined, and the measure of mental health used. For women, infrequent participation in active sports consistently predicted mental health problems, but was not important for men. In young men, no association was found between health habits and mental health. Dietary practices and mental health generally showed a negative association, especially among men, for whom those reporting good practices had a significantly higher risk of nervous breakdown than those reporting poor practices. (Simonsick. Am J Prev Med, 1991;7:425-37.)

- Several studies have demonstrated a strong association between physical and psychiatric disorders in the general population. An examination of the prevalence of mental and physical disorders, singly and in combination, among persons 18 years of age and older resident in the Baltimore Epidemiologic Catchment Area supports and extends these findings. Over 40 percent reported a physical disorder and 23 percent had a recent mental disorder. Over 25 percent of persons with a physical health problem had a mental disorder and about half of persons with a mental disorder also reported a physical disorder. The prevalence rates of mental disorder given a physical condition are particularly high for males, non-whites, persons 18 to 34 years, the unmarried and persons residing in female headed households. Substantial proportions of persons with physical and/or mental disorders reported no health service utilization six months prior to interview. This analysis highlights the high prevalence of physical and mental disorders and their co-occurrence in the community and the significant unmet need for primary and mental health care. (Kramer M, Simonsick E, Lima B, Levav I. Primary Health Care and Psychiatric Epidemiology, London: Rutledge, 1992;71-96.

CONTRACT

Name and Number: University of Iowa (N01-AG-0-2106)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $310,578

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1989-93) will be based on the use of the National Death Index for mortality and the Health Care Financing Administration's Medicare data for morbidity.

Major Findings: Several theoretical perspectives on division of household work in retirement are discussed and empirical findings on division of household work after retirement are reviewed. Future research needs and implications for practice are also discussed. Information is provided on what to expect in retirement for couples who are planning their retirement and for couples who are already retired (ref. 1).

The major research that has appeared in the past decade on work and retirement in rural America are reviewed as well as new information about retirement. The adequacy of current information available to the aging network is assessed and projections about what trends to expect in the next 5 years are made. Suggestions are made for the aging network regarding policy, resource allocation, and education and training that should help overcome barriers of isolation, economic deprivation, lack of an adequate human service infrastructure, and lack of a trained labor pool that affect provision of programs and services to retired and preretired rural elders (ref. 2).

Three approaches are suggested in a discussion of medical conditions and the risk of motor vehicle accidents (MVAs) using principles derived from geriatrics, gerontology, and health

services research. The medical model uses discrete diseases as risk factors, but despite its utility and appeal, this model does not sufficiently characterize health and functional status to be the sole approach, and future investigative efforts should at least consider severity of illness concepts. The functional status model emphasizes a functional and physiological approach to health status, but despite its successful application in geriatric assessment and its investigative promise, functional status can be difficult to measure and has not been validated in relation to MVA risk. Finally, a behavioral model is considered and within that framework the relation of depressive and other emotional illnesses are discussed as well as social vitality to driving patterns and MVA occurrence (ref. 3).

Publications

1. Dorfman LT. Couples in retirement: Division of household work. In: Szinovacz M, Ekerdt D, and Vinick B. eds. Families in retirement. Newbury Park: Sage Publications, 1992;159-173.

2. Dorfman LT. Work and retirement in rural America. In: Bull N. and Bane S. eds. The future of aging in rural America: Proceedings of national symposium. Kansas City: National Resource Center on Rural Elderly, 1992;117-131.

3. Wallace RB, Retchin SM. A geriatric and gerontologic perspective on the effects of medical conditions on older drivers. Human Factors 1992;34:17-24.

CONTRACT

Name and Number: The Johns Hopkins University School of Medicine (N01-AG-1-2112)

Title: Women's Health and Aging Study

Date Contract Initiated: July 1, 1991

Current Annual Level: $1,678,933

Objectives: The overall goal of the study is to understand the causes and course of physical disability (defined as a deviation or alteration in normal functional performance) in older women living in the community. This will be accomplished by (1) screening a representative population of community-dwelling older women to recruit a study cohort of women with moderate to severe dependence in physical functioning, (2) characterizing prevalent diseases and conditions in members of this cohort and assessing the impact of these conditions on physical function, and (3) following the cohort prospectively for a period of 3 years to evaluate change in functional status.

Methods Employed: A household screening assessment of approximately 5,400 community-dwelling older women living in Baltimore city and county will be used to select 1,000 women who have the greatest degree of disability. At baseline these women will receive a comprehensive interview, a physical examination, and various diagnostic tests. Those electing to participate in the study will receive follow-up assessments at 6-month intervals. A small subset of the study cohort will receive intensive followup over a 6-month period, with weekly contacts to assess short-term changes in functional status.

Significance to Biomedical Research: This study has a number of aspects that make it unique as an epidemiologic study and add substantially to previous work done on disability in older populations. Epidemiologic studies in general have tended to study factors leading to the onset of incident disease, with little study of subjects once disease occurs. This study will examine women who already have disease and disability, attempt to understand the diseases underlying that disability and then prospectively evaluate the course of disability and how the underlying diseases as well as health habits, psychological, cognitive, social and other factors affect that course. Unlike previous epidemiologic research on disability, this study will intensively evaluate diseases and physiologic dysfunction that are associated with disability.

Proposed Course: At baseline, data will be collected on the major diseases and conditions of aging to ascertain their presence, severity, and impact on disability. The cohort will be followed for 3 years, with in-home follow-up assessments every 6 months to evaluate change in disease and functional status. Members of the cohort will also be monitored for hospitalization, nursing home admission, the use of home health care, and mortality.

Two rounds of pretests were carried out during January and February 1992, to assess (1) the time required to administer the questionnaires and examination, including timings for

individual subsections, and (2) to assess the acceptability of the interview and examination to participants. The volunteers were similar in age and disability status to those who will be eligible for the study. These individuals participated in the screener, the baseline interview, and the nurse practitioner examination. Based on these pretests, instruments were modified and finalized prior to submission for OMB clearance in March 1992.

The clinical investigators on the study team have developed algorithms specifying criteria for determining both presence and severity of disease. The algorithms take into account information obtained by respondent report (from the interviews), by clinical assessment (from the home examination), and from medical records (through the surveillance activity). The algorithms cover the following diseases and conditions: cancer, angina, coronary heart disease, congestive heart failure, status post myocardial infarction, intermittent claudication, fractures, osteoarthritis of hands, hips, and knees, rheumatoid arthritis, disc disease, stroke, peripheral neuropathy, Parkinson's Disease, pulmonary disease, diabetes mellitus, and vision impairment.

The process of developing and revising the algorithms is nearly complete. Work has begun on operationalizing them with regard to the specific data elements required, from what sources they will come, and how they will be incorporated into the data management system and compiled for use in the adjudication process, in which clinician investigators will make judgements on the presence and severity of diseases and the diseases directly responsible for the disability observed in study participants.

The HCFA Medicare enrollment files, the sampling frame for this study, have been received. The total number of women aged 65 and over in the geographic area is 32,539. Participants in the Current Beneficiary Study (N=16) and in the Senior Health Watch project (N=2258) were removed, leaving a sampling frame of 30,266, a number very close to that estimated in the proposal. The age and race distributions are also similar to those estimate earlier, though there is a somewhat higher than expected proportion of black women (21 percent as compared to the estimate of 16 percent). The samples for the first replicate were drawn in mid-July.

The current schedule for the project calls for the screening and baseline interviewing to begin October 1992. Development of procedures and materials for training is underway. The nurse practitioner training and interviewer training will begin in September.

The contract for the Women's Health and Aging Study has been modified to specify the use of computer-assisted personal interviewing (CAPI) in the baseline and follow-up interviews. Specifically, this entails supplying each interviewer with a notebook computer which is used to enter responses to questions directly. While there are additional costs associated with this process, related primarily to programming and equipment leasing, there will be improvements in the quality of the information obtained and in the timeliness with which the data will be available for analysis. In particular, CAPI will facilitate the linkage of information between interviewing waves, so that appropriate follow-up questions can be asked based on information obtained in previous interviews.

Asia-Pacific Office

The Asia-Pacific Office plans and conducts a variety of epidemiological studies predominantly among Pacific and Asian populations, and coordinates new international and cross-cultural initiatives aimed at investigating risk factors for dementia, diseases, disabilities and other concomitants of aging.

The Asia-Pacific Office (APO) was established in August 1990, with the assignment of its Chief (Dr. Lon White, formerly Chief of the Epidemiology Office) to Honolulu. The Office is based at Kuakini Medical Center in space provided by that institution through a National Heart, Lung, and Blood Institute (NHLBI) contract in support of the Honolulu Heart Program. The APO serves as the nucleus of the Asia-Pacific Office for Research on Aging (APORA), an informally constituted and named multi-agency entity currently comprised of representatives from the NIA (Dr. White), the National Center for Nursing Research (NCNR) (Dr. Carolyn Murdaugh), and the Department of Veteran's Affairs (DVA) (Dr. George Webster Ross). The primary activities of the APORA, as it operates at present, are related to the conduct, direction, and utilization of the Honolulu-Asia Aging Study (HAAS) as a scientific research project.

Honolulu-Asia Aging Study

The HAAS is conducted through a multi-institutional cooperation among personnel of the NIA, the prime contractor (Kuakini Hospital), sub-contractors (University of Hawaii and others), the DVA, NCNR, and the NHLBI. This federal-contractor interaction is working smoothly and effectively. This is in part a reflection of complementarity of the expertise of the federal workers, and that of the contractor's and subcontractors' staffs.

The basic purpose of the HAAS is to utilize the HHP as a resource for research on dementia, and to conduct the study so as to allow comparisons of results with those generated by parallel studies in other Asian-ancestry populations. One aspect that makes the HHP such a promising resource is the existence of a great deal of information on the participants' health, diets and blood measures, all gathered prospectively and therefore all unbiased for the development of dementia. The second aspect is related to the Japanese ancestry of the cohort.

Summary of the Year's Activities.

The major activities of the past year continued to be directed toward data collection in the Honolulu-Asia Aging Study (HAAS). Funding for the HAAS is accomplished by an interagency agreement between the NIA and the NHLBI to support an aging and dementia supplement to the NHLBI's Honolulu Heart Program (HHP) contract. The HHP is a longitudinal study of heart disease and stroke that began more than 25 years ago and is directed by the NHLBI through a research contract with the Kuakini Medical Center. The principal scientific goals of the HAAS involve definitions of rates, patterns, correlates, and predictors of cognitive decline, dementia, Alzheimer's disease (AD), and cerebrovascular dementia. In addition, a substantial amount of

the research is related to methodological issues, including the development and application of instruments for use in cross-cultural studies, issues related to sensitivity and specificity of methods and instruments in such studies, and validation of diagnoses (to examine endpoint misclassification problems).

Of the approximately 8,000 Japanese-American men who participated in the first HHP examination, approximately 5,000 are alive today. It is expected that approximately 4,200 will be examined during the current (4th) HHP examination, now expected to end in September 1993. More than 2,000 have been examined to date. Approximately 20 to 25 minutes of the baseline 4th examination is devoted to NIA data collection, including administration of the Cognitive Assessment and Screening Instrument (CASI) and a questionnaire to elicit information concerning possible risk factors for dementia. Approximately 20 percent of those seen for the baseline Cycle 4 Examination are invited back for a further examination, the algorithmic dementia evaluation (also referred to as Module II Level 1), consisting of re-administration of the CASI, a test of reaction time, a test of manual dexterity, the Center for Epidemiologic Studies-Depression (CES-D) scale, a brief hearing test, brief vision test, a standardized neurological examination, and interview with the caregiver -- usually the spouse, occasionally a son, daughter, or other significant person. Approximately 40 percent of those who complete the algorithmic evaluation are invited back a third time for a clinical dementia evaluation, corresponding generally to a CERAD diagnostic evaluation for dementia. More than 400 participants have returned for the algorithmic evaluation, and approximately 150 for a clinical evaluation. An intensive effort has been devoted to recruitment of participants and their caregivers in an effort to maximize response rates. Although all phases are slightly behind schedule, the project is generally progressing well, and expected to be successful.

A major accomplishment of this fiscal year has been the establishment of a system for obtaining autopsies. Autopsy and neuropathologic validation of our cases and (thereby) of the methods used to diagnose dementia in the HAAS will be of extreme importance. Over the past decade autopsy rates in HHP members have fallen from approximately 50 percent to 1 to 2 percent. Our goal is to increase rates of autopsy in those individuals diagnosed in the HAAS as demented to more than 70 percent, to more than 50 percent in the non-demented participants who have received Module II evaluations, to 20 percent among participants who have received a Module I examination but were not selected for Module II, and to 10 percent in all other deaths among HHP cohort members. Among HHP participant deaths in recent years, about 35 percent have occurred at Kuakini Hospital. Our current efforts to increase autopsy rates are aimed at patients hospitalized at Kuakini Hospital. As a result of intensive efforts, we have been able to obtain consent for autopsy in about 40 percent of deaths occurring at the hospital over the past 3 months (8 of 18 deaths). Efforts are continuing to increase this rate further. At the same time we have begun negotiations with the Honolulu medical examiner's office aimed at increasing rates of autopsy for out-of-hospital deaths. Although an autopsy protocol has been defined, several specific aspects, including forms and methods for recording observations, remain to be defined. To address these issues, Dr. William Markesbery (Lexington) and Dr. Mark Sumi will visit Honolulu as contract consultants during early September.

As mentioned, current plans call for completion of the 4th Cycle, baseline (Module I) examination, in September 1993. The NHLBI is considering the conduct of a 5th examination

cycle. NIA's portion of the 5th examination interview will have to be determined. Plans have been developed to expand the content of the 1993 to 1995 exam cycle to include an evaluation of hearing, language, and communication abilities in Japanese and English, and to obtain magnetic resonance imaging of the brain on all participants and possibly to measure fat-free mass.

In June the Asia-Pacific Office hosted the Sixth Japan-U.S. Workshop on Methods for Cross-National Dementia Research. The purpose was to provide a forum for planning collaborative research projects, and to present our collaborators with a detailed review and description of methods currently in use in Honolulu and Seattle. The 4-day workshop was attended by 13 researchers from Japan, 6 from Taiwan, 1 from the People's Republic of China, 10 from Seattle, 4 from Guam, 1 from Los Angeles, all of the HAAS investigators, and a number of observers. The workshop accomplished both goals with excellent success.

In addition to directing the HAAS, the mission of the Asia- Pacific Office includes collaborating with, assisting, training, and generally encouraging the development of other researchers in Pacific region and Asia. Assistance, training, and/or advice has been provided to several researchers in Honolulu, including Dr. David Morens (grant application for research on Parkinsonism in HHP cohort members was recently funded), Andy Grandinetti (graduate student to be working with materials from the HHP and HAAS), Dr. Gilbert Wergowski (recently a University of Hawaii Geriatrics Fellow; paper presented at the 1991 Gerontology meetings), and Dr. Max Vercruyssen (currently applying for an NRSA fellowship). Dr. Paul Bogdan (Department of Medicine, University of Hawaii) is currently receiving training in HAAS methods, and is expected to participate in future analytic activities. Special assistance was provided to the Micronesian Health Study (Guam) and its Principal Investigator, Dr. Leonard Kurland, related to methods that might be used in research on dementia on Guam. Dr. George Webster Ross, the HAAS project neurologist, was encouraged to seek special training in epidemiology and did so by attending a summer epidemiology training program at the University of Michigan.

Coordination with the NI-Hon-Sea-Tai Project.

The Ni-Hon-Sea-Tai project is a coordinated series of epidemiologic studies on dementia that includes: (1) HAAS, (2) the KAME project, a closely coordinated study conducted among Seattle's older Japanese-American population (funded as an NIA grant), (3) a survey in the Adult Health Study Cohort of the Radiation Effects Research Foundation (REFR) (Hiroshima), (4) a survey to be conducted in Kawasaki City (St. Marianna's University and the Tokyo Metropolitan Institute of Gerontology), and (5) surveys to be conducted in Taipei (National Taiwan University and the Veteran's General Hospital). The project was organized by the Asia-Pacific Office and is continued as a shared effort by the cooperating scientists. A central goal is to develop and use methods sufficiently alike so that comparisons of rates and patterns of dementing illnesses across sites would be possible.

Coordination with the Hiroshima study was facilitated by establishment of an interagency agreement with the Department of Energy, through which funds were made available to the RERF for work related to our collaborative studies on dementia. A large part of these funds

were used to support the participation of Japanese scientists in the Sixth Workshop (vide supra). Close cooperation with the KAME project was accomplished by frequent telephone communication and by visits of KAME researchers to Honolulu as consultants to the HHP contract, and to attend the Sixth Workshop. Collaboration with Taiwanese researchers was facilitated by professional services contracts with two investigators from Taiwan National University and the University of Michigan. Informal collaborative relationships with Taiwan researchers from Kaohsiung University and the Veterans General Hospital were established during the Workshop. This project continues to be based on informal agreements among the collaborating researchers and has no specific support other than that available through the HAAS contract, the resources of EDBP, the KAME grant, and local funds provided occasionally by the cooperating centers. Despite this lack of formal support, the project is progressing extremely well and it is likely that parallel studies using comparable methodologies will be conducted at two sites in the U.S., one or two sites in Japan, and at least two sites in Taiwan.

Preliminary Results

It appears that the prevalence of dementia in the cohort will be in the range of 7 to 8 percent, with about 45 percent of the cases being attributed to AD and 35 percent to cerebrovascular causes. This overall prevalence estimate is slightly lower then that observed in the East Boston EPESE population and similar to the prevalence in the Framingham cohort. However, the proportion of cases attributed to vascular causes appears to be higher than has usually been observed in American surveys. These data are preliminary, being based on 50 cases of dementia identified by the HAAS project neurologist.

Travel and Presentations

In October and November 1991 Dr. White traveled to Yokohama, Japan as a speaker and co-chair of a Symposium on Psychiatric Epidemiology at the 4th Asia/Oceania Regional Congress of Gerontology. His presentation was entitled, "The Ni-Hon-Sea Dementia Project - Description and Progress Report."

In December 1991, Dr. White presented a lecture on Alzheimer's disease and related dementing diseases at a University of Hawaii Geriatric Board Update and Training Course.

In January 1992, Dr. White delivered a lecture on Epidemiologic Research on Aging to the University of Hawaii Center on Aging, Seminars on Aging Program.

In February 1992, Dr. White participated in a meeting on Neurodegenerative Disorders in the Western Pacific Region, held in Guam and organized by the University of Guam and the Mayo Clinic.

In March 1992, Dr. White traveled to Hiroshima as a member of the Future Planning Committee for the Radiation Effects Research Foundation.

In May 1992, Dr. White traveled to Kauaii, Hawaii, as a speaker at the NHLBI-sponsored Japan-U.S. Conference on Cardiovascular Diseases, giving a presentation on stroke and dementia in the Honolulu Heart Program.

In June 1992, the Asia-Pacific Office hosted the Sixth Japan-U.S. Workshop on Methods for Cross-National Dementia Research; the entire program was organized and chaired by Dr. White.

In July 1992, Dr. White presented a review of research on olfaction to the Honolulu Neuroscience Club.

On September 8, 1992, Dr. White is scheduled to deliver a presentation on Epidemiology and Risk Factors for Alzheimer's disease at an Update on Alzheimer's Disease, sponsored by the University of Hawaii and the Honolulu Alzheimer's Association.

On September 23, 24, and 25 Dr. White is scheduled to participate as a member of the KAME project scientific advisory committee, meeting in Seattle.

Awards

Dr. Lon White received the PHS Commendation Medal for inaugurating cross-cultural epidemiologic research on Alzheimer's disease by creating an Asia-Pacific Office and starting the Honolulu-Asia Aging Study.

Research Highlights FY92

- We determined the prevalence of dementia and probable senile dementia of the Alzheimer type (SDAT) for biennial Exam 17 of the Framingham cohort (1982/1983). The prevalence of dementia was 30.5/1,000 for men and 48.2/1,000 for women and increased with advancing age. Cases of probable SDAT constituted 55.6% of all dementia cases. The prevalence of SDAT was 11.7/1,000 for men and 30.1/1,000 for women and also increased with advancing age. Prevalence of dementia and probable SDAT were greater for women than men. The female:men ratio of prevalence for cohort members 75 years of age and older was 1.8 for all cases of dementia and 2.8 for cases of probable SDAT. (Bachman, Wolf, Linn, Knoefel, Cobb, Belanger, D'Agostino, White. Neurology, 1992;42:115-119.)

PROJECT NUMBER Z01 AG 07040 02 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Honolulu Aging Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Lon R. White, M.D., M.P.H., Chief, Asia-Pacific Office, EDBP
Robert Garrison, M.D., Chief, Field Studies Branch, EBP, NHLBI
G. Webster Ross, M.D., Project Neurologist, DVA
Carolyn Murdaugh, Ph.D., Senior Investigator, NCNR

COOPERATING UNITS (if any)

National Heart Lung and Blood Institute (NHLBI)
National Center for Nursing Research (NCNR)
Department of Veterans Affairs

LAB/BRANCH

Asia-Pacific Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.9	.85	.05

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects — X (b) Human tissues — O (c) Neither

O (a1) Minors

X (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The National Institute on Aging (NIA) supplements a research project (the Honolulu Heart Program) sponsored by the NHLBI and supported through an NHLBI contract with Kuakini Medical Center in Honolulu, Hawaii, to allow for research on aging and dementia among study participants. The Honolulu Heart Program is a prospective study of cardiovascular diseases of American men of Japanese ancestry born from 1900 to 1919 and living on the island of Oahu in 1965. This study will focus on aging, with the emphasis on Alzheimer's disease and multi-infarct dementia. Approximately 5,200 men aged 70 to 90 will be contacted over a 2-year period. Data collection method and procedures involve examination and interview of individual participants, including blood drawing and testing.

Preliminary results indicate that the prevalence of dementia in the cohort will be in the range of 7 to 8 percent, with about 45 percent of the cases being attributed to AD and 35 percent to cerebrovascular causes. This overall prevalence estimate is slightly lower then that observed in the East Boston EPESE population and similar to the prevalence in the Framingham cohort. However, the proportion of cases attributed to vascular causes appears to be higher than has usually been observed in American surveys. These data are preliminary, being based on 50 cases of dementia identified by the project neurologist.

PROJECT NUMBER Z01 AG 07070 01 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

March 5, 1992 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiologic Studies on Brain Aging in Japan and the United States

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Lon R. White, M.D., M.P.H., Chief, Asia-Pacific Office, EDBP, NIA
Richard J. Havlik, M.D., M.P.H., Associate Director, EDBP, NIA
Harry Pettengill, Ph.D, Deputy Assistant Secretary for Health, DOE
Robert Goldsmith, Ph.D., Director, Office of Epidemiology and Health Surveillance, DOE

COOPERATING UNITS (if any)

U.S. Department of Energy

LAB/BRANCH

Asia-Pacific Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.01	

CHECK APPROPRIATE BOX(ES)

O (a) Human subjects — O (b) Human tissues — X (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Ni-Hon-Sea-Tai project is a coordinated series of epidemiologic studies on dementia that include a survey in the Adult Health Study Cohort of the Radiation Effects Research Foundation (REFR) in Hiroshima. The project was organized by the Asia-Pacific Office and is continued as a shared effort by cooperating scientists in Seattle, Kawasaki City, Taipei, Hiroshima, and Honolulu. A central goal is to develop and use methods sufficiently alike so that comparisons of rates and patterns of dementing illnesses across sites would be possible.

Coordination with the Hiroshima study was facilitated by establishment of an interagency agreement with the Department of Energy, through which funds were made available to the RERF for work related to our collaborative studies on dementia. A large part of these funds were used to support the participation of Japanese scientists in the Sixth Japan-U.S. Workshop on Methods for Cross-National Dementia Research.

The project is progressing well and it is likely that parallel studies using comparable methodologies will be conducted at two sites in the U.S., one or two sites in Japan, and at least two sites in Taiwan.

Geriatric Epidemiology Office

The Geriatric Epidemiology Office plans and conducts studies and analyses of data in the areas of biomedical, genetic, metabolic, and gerontological issues. The Office Chief will develop programs to integrate biomarker research into epidemiology of aging; define hypotheses and design projects to investigate specific questions related to metabolic/nutritional issues, chronic inflammation, and comorbidity as these areas relate to functional outcomes and mortality in older persons.

The Geriatric Epidemiology Office was established in the Epidemiology, Demography, and Biometry Program with the recruitment of an Office Chief, Dr. Tamara Harris, who joined the program in November 1991. Dr. Harris is a board-certified internist with additional training and certification in Geriatric Medicine. She also holds a Master of Science degree in Human Nutrition and a Master of Science degree in Epidemiology. Dr. Harris was recruited from the National Center for Health Statistics, where her epidemiologic work focused on nutrition-related risk factors for morbidity and disability in older persons and the component for older persons from the National Health and Nutrition Examination III Survey.

Dr. Harris has devoted time over this year to planning and organizing activities of the Geriatric Epidemiology Office, implementing new projects, and continuing and initiating analytic projects. These activities are summarized below by the projects to which they apply.

1. Assessment of the relation of chronic disease burden, weight loss and immune activation

An augmentation of the Framingham Heart Study was implemented. The purpose was to investigate the association between chronic disease burden, weight loss and inflammation in older persons. An interagency agreement was initiated with the USDA Human Nutrition Center on Aging at Tufts Medical School in Boston to examine cytokine levels in mononuclear cells, levels of inflammatory proteins, and associated other biochemistries including antioxidant levels and lipids. The collection of specimens began early April of 1992; methodologic issues regarding whether time for transport to the laboratory could affect measurement are being assessed.

2. Determinants of Healthy Aging

Dr. Harris has worked with Dr. Stefania Maggi, Chief, Special Program for Research on Aging (SPRA), World Health Organization (WHO), over the past year on the continued development and refinement of the protocol for the study. She attended a meeting in Geneva, Switzerland, at which time the WHO SPRA Program endorsed the project. With Dr. Maggi, Dr. Harris has planned two workshops for this project. The first, to take place September 14, will focus on performance measures for use in international comparative epidemiologic studies in aging; the second will focus on high priority physiologic measures for international comparative epidemiologic studies in aging, including laboratory tests. In

addition, support is being offered for small pilot tests of both a physical performance battery and the physiologic measures in several developing countries which are to be included in the study.

3. WHO Anthropometry

Working with Dr. Lenore Launer of the Free University in the Netherlands, Dr. Harris initiated a study of the distribution of weight, height, and body mass index in selected populations of older persons. These data will be presented at the Steering Committee meeting convened by Dr. Phyllis Eveleth for October 1992.

4. Measurement of insulin-like growth factor in cycle 22 of the Framingham Heart Study

To investigate the distribution and concomitants of insulin-like growth factor (IGF), a contract was let to Endocrine Sciences to perform this assay in blood from the current cycle of the Framingham Heart Study. The values obtained will be examined in relation to past levels of body fat and physical activity; in addition, a longitudinal study of change in IGF1 levels over time will be undertaken comparing these data with values from an earlier examination cycle. Furthermore, these IGF1 levels will be examined in relation to osteoarthritis and osteoporosis being ascertained at this current examination. In addition, on approximately 90 percent of the sample, both bioelectric impedance and dual x-ray absorptiometric measures of body composition will be available for correlation with IGF1 levels.

5. Longitudinal change in cholesterol and HDL in relation to inflammatory proteins

In several cross-sectional studies, total cholesterol tends to decline in old age associated with decline in health status and increasing disability. However, HDL levels tend to remain constant. To investigate this in a longitudinal study and to ascertain the association of this decline with inflammation, a contract was initiated with the Cardiovascular Health Study site at Bowman Gray School of Medicine. Serum cholesterol measurements are available for four separate examinations; this contract will cover the cost of the HDL measurements and the measurement of C-reactive protein, an inflammatory marker.

6. Study of longitudinal weight change in rhesus monkeys

Weight tends to increase with age. There is some question whether increased morbidity in old age may be linked to weight gain at younger, as opposed to older, age. There is little data available from humans on weight over the lifespan and it has been suggested that animal models may be appropriate. Morgan Island, South Carolina, is a colony of free-living freely feeding rhesus monkeys maintained as a breeding colony by the FDA. A contract was initiated with the manager of the colony to create a computerized data set with all

information on age, weight, and any health information for aged animals who have lived in the Morgan Island colony. These data will be used to examine natural history trends with age for weight in these animals.

7. Consultation, Collaboration and Other Professional Activities

Since joining the NIA, Dr. Harris has participated in the following activities:

Medical Care Surveys - Provided consultation regarding the feasibility of a National Health Care Survey Patient Follow-up

Office of Prevention - Provided consultation for the Women's Health Initiative observational cohort; participated in the SEG for the coordinating center and the contract sites.

WHO Committee on Anthropometry in Older Persons - Work on developing population epidemiology issues related to anthropometry.

Nutrition Office - Attended meetings and prepared conference proposal for NIA-sponsored workshop on weight, weight change and health outcomes in old age.

NIH Nutrition Coordinating Committee - Alternate representative.

Cardiovascular Health Study (CHS) - Dr. Harris attended the Steering Committee meeting in Washington in January. She was asked to participate as a co-author on several CHS publications, particularly those dealing with disability and weight. Dr. Harris organized a meeting with Dr. Lon White (by speaker phone), Drs. Barry Horwitz, Charles DeCarli and Judith Salerno of the LNS Brain Aging and Dementia Section; Dr. Terry Radebaugh of NNA; Dr. Patty Davis of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD); and Drs. Teri Manolio, Peter Savage and Nicholas Bryan of the CHS project to discuss the MRI studies in CHS. Subsequently, Dr. Harris met with Dr. Radebaugh and Dr. Neil Bucholtz to investigate mechanisms under which additional focused studies using the MRI data could be initiated by investigators in CHS.

QRB for NIDCD - Dr. Harris was a member of the committee which interviewed candidates for the position of Epidemiologist at the National Institute of Deafness and Communication Disorders.

USDA Human Nutrition Center on Aging - Planning Committee, Nutrition Assessment in Elderly Conference.

Continuing collaborative associations:

NCI - AARP Dietary Survey - Cohort study of several hundred thousand persons age 50 to 69 for dietary factors in cancer incidence.

NHLBI - Data Monitoring Board of the CRISP Study

National Center for Health Statistics

National Health and Nutrition Examination Survey III - Continue to advise regarding component for older persons; participation of older respondents; issues of science with regard to older persons; planning and augmentation of followup of NHANES III cohort; nonresponse evaluation of older persons; and beginning analytic projects.

International Collaborative Effort on Aging - Continue to participate as member of group gathering information on hip fractures from disparate international sources; manuscript submitted and under revision regarding comparison of hip fracture rates in U.S. and Hong Kong.

Longitudinal Study on Aging (LSOA) II - Continued to be involved in planning and discussions for LSOA II especially with regard to incorporation of the NHANES III cohort of older persons into this longitudinal study.

Health Care Financing Administration (HCFA) - Technical Advisory Panel for the Current Beneficiaries Study and Advisory Panel for the Post-hospitalization Outcomes Study.

Nutrition Screening Initiative - Gave technical assistance to the scoring of the first level screening instrument by modelling the scoring using data from the NHEFS. Manuscript based on this is in progress with Dr. Barbara Posner of Boston University.

Dr. Harris consulted with Dr. L. de Groot and Dr. W. Van Stavaren of the SENECA-Euronut Study.

Dr. Harris served as a manuscript reviewer for the following journals: the New England Journal of Medicine, Journal of the American Geriatrics Society, Journal of Gerontology, American Journal of Epidemiology, Aging, and the Journal of General Internal Medicine.

Dr. Harris was a member of the review panels for the NIA Claude Pepper Older Americans Independence Centers.

Dr. Harris reviewed abstracts for the Gerontological Society, Clinical Medicine section and the American Geriatric Society.

American Geriatrics Society, coordinated Health Services Research abstract review and selected abstracts for paper presentations.

9. Analytic Efforts

Established Populations for Epidemiologic Studies of the Elderly (EPESE):

Body mass index and risk of mortality. Effect of health status. Analysis with Kathy Losonczy and Eleanor Simonsick.

Correlates of HDL cholesterol in older persons. Analysis with Robert Wallace and Marcel Salive. While cholesterol levels tend to decline cross-sectionally in older persons correlated with increasing levels of poor health and disability, HDL cholesterol levels tend to be maintained. This analysis will examine HDL cholesterol levels in the EPESE study in relation to traditional determinants of HDL such as exercise, body mass index, and alcohol intake. In addition, HDL will be related to functional status and other measures of health status and related to level of total cholesterol.

NHANES I Epidemiologic Followup Study (NHEFS):

Dr. Harris in collaboration with Dr. Suzanne C. Ho of the Department of Community and Family Medicine, the Chinese University of Hong Kong, Shatin, Hong Kong, and Drs. Jennifer Madans and Jacob J. Feldman of the Office of Analysis and Epidemiology, NCHS submitted for publication a paper entitled, "Body mass index, mortality and disability among non-smoking elderly women: A comparative study between Hong Kong and the United States." To address the relationships between weight and mortality and weight and disability in old age, data on women aged 70 or older were compared from two studies of health in old age: one from the U.S. and one from Hong Kong. The mean periods of follow up are 32 months and 37 months respectively. In order to eliminate confounding by smoking, the data sets were restricted to elderly women who were never smokers. Proportional hazards analysis shows an inverse relationship between age-adjusted mortality and body mass index in both elderly populations. Similar findings were observed after controlling for health and social conditions, and also after excluding subjects with disability at baseline. Disability at baseline and at followup was also highest among subjects belonging to the body mass index group less than 20.4 and the findings were consistent in both populations. These results were accepted for publication in Age and Nutrition.

Is proteinuria on dipstick urine a reasonable screening test for significant health problems? Analysis of data from urinanalysis from NHANES I over 14 years of followup examining the relation of trace or more proteinuria with incident renal disease and heart disease, as well as all-cause and cause-specific mortality.

What aspects of nutrition-related behaviors predict mortality in an older cohort? This analysis, with Barbara Posner of Boston University, examines the results of modelling the scoring of the Nutrition Screening Initiative instrument in the NHEFS for men and women and the relation of total score and individual items to mortality.

Relationship of weight and weight change to risk of coronary heart disease in older persons. An analysis which shows that current measured weight is not associated with risk of CHD, but past weight is. The main reason that current weight is not a risk factor for CHD is that older heavier persons have lost much weight. It is unclear whether the risk of CHD relates to the earlier overweight or to factors associated with weight loss which may increase the risk of CHD.

Weight, weight change and comorbidity as risk factors for disability. This paper examines risk of incident mobility-related disability in the NHEFS in relation to current weight, past weight and weight change in older and younger women. With Dr. Lenore Launer of the Free University.

Gravidity and risk of CHD. This manuscript has been submitted for publication and shows that, even controlling for known cardiovascular risk factors, data from NHEFS and the Framingham Heart Study suggest that women with six or more pregnancies are at increased risk for CHD. With Dr. Roberta Ness of University of Pennsylvania.

Weight and well being in younger women. This analysis, initiated by Dr. Catherine Rumpel at the NCHS, examines the relationship of weight change to score on the General Well-Being Scale.

Body mass index and hip fracture. This analysis, with Dr. Anne Looker of the National Center for Health Statistics, shows that the association of low weight with risk of hip fracture is seen primarily in women who have lost a lot of weight. Among those who were relatively weight stable, there was no association with weight for hip fracture.

9. Talks and Presentation

November 1991, symposium presentation, Gerontological Society of America, "Sick, old or what? Modification of the Low Cholesterol-Mortality Association by Time, Age, and Activity Level."

December 1991, invited plenary presentation, First European Congress on Nutrition and Health in the Elderly, "The National Health and Nutrition Examination III for older persons".

December 1991, abstract presentation, First European Congress on Nutrition and Health in the Elderly, "Issues in Nonresponse of Older Persons in NHANES III".

December 1991, abstract presentation, First European Congress on Nutrition and Health in the Elderly, "Weight and incidence of coronary heart disease in older women: The NHANES I Epidemiologic Followup Study".

December 1991, invited participant, Food and Nutrition Board, Institute of Medicine. Reconsideration of RDA's for Older Persons.

January 1992, invited presentation, Conference on Cardiovascular Health and Disease in Women, "Relationship between cardiovascular disease and other comorbidities".

March 1992, invited presentation, Department of Health and Human Services Nutrition Symposium, "Heterogeneity of health in the older population and its effect on nutritional status."

March 1992, invited presentation, National Center for Health Statistics Seminar, "The National Health and Nutrition Examination III for older persons".

March 1992, invited participant, WHO Research Program on Aging, Steering Committee, Determinants of Healthy Aging.

April 1992, invited presentation, Advances in Health Care for Older Women: Menopause and Beyond, "Risk Factors and Risk Factor Interventions for Cardiovascular Disease in Women."

April 1992, invited presentation, NIH Epidemiology Training Program, "An overview of issues related to analysis of data on older persons."

May 1992, abstract presentation, American Federation for Clinical Research, "Weight, weight change and risk of coronary heart disease in persons aged 70-86 years of age: The NHANES I Epidemiologic Followup Study."

June, 1992, invited presentation, Nutrition and Aging meeting sponsored by Centre de Recherches International pour la Sante at NIH, "NHANES III: Methodology and issues relevant to the health of older persons."

July 1992, invited presentation, Gerontology Research Center, NIA, "Body Weight, weight change, risk of death and disability in older persons."

September 1992, invited presentation, American College of Cardiology meeting on Inclusion of Elderly Individuals in Clinical Trials: Cardiovascular Disease and Cardiovascular Therapy as a Model, "How might heterogeneity in the older population increase the risk of J-shaped curves in clinical trials?"

10. New Staff

An advertisement was placed and solicited several good applicants for an epidemiologist position. Unfortunately, this position was caught in the freeze and alternative mechanisms are being explored.

However, Dr. Lenore Launer of the Free University in The Netherlands was a Guest Researcher from May 26 to June 22. Dr. Launer is a Ph.D. nutritionist who completed the Epidemiology Training Program before moving to the Netherlands. She and Dr. Harris worked on the WHO Anthropometry Report. In addition, she and Dr. Harris carried out an analysis of data from the NHANES I Epidemiologic Followup Study on the relationship of current weight, past weight and weight change on the risk of disability.

Marian Parrott has also joined the Geriatric Epidemiology Office under an Intergovernmental Personnel Act (IPA). Dr. Parrott is physician-geriatrician who is a full-time faculty member in the Geriatric Medicine group of the George Washington University School of Medicine. Dr. Parrott is spending 40 percent time working with Dr. Harris for the next year. Her projects will examine risk factors for prostatectomy in the NHANES I Epidemiologic Followup Study.

An Epidemiology Training Program trainee, Dr. John Sorkin also joined the Geriatric Office. Dr. Sorkin, who has been a Staff Fellow with Dr. Reubin Andres at the GRC, will begin his course work at The John Hopkins University School of Hygiene and Public Health this September and will join EDBP in July of 1993.

In addition, Dr. Harris continues to partially supervise Dr. Catherine Rumpel, an NCHS participant in the Epidemiology Training Program. Dr. Harris also serves as a member of the thesis committee for Yoachim Ostchega, an NCHS employee who is enrolled in a doctoral program at the University of Maryland.

Research Highlights for FY92

- The relationship of low serum cholesterol and mortality was examined in data from the NHANES I Epidemiologic Followup Study (NHEFS) for 10,295 persons aged 35-74, 5,833 women with 1,281 deaths and 4,462 men with 1,748 deaths (mean followup = 14.1 years). Serum cholesterol below 4.1 mmol/l was associated with increased risk of death in comparison with serum cholesterol of 4.1-5.1 mmol/l (relative risk (RR) for women = 1.7, 95% confidence interval (CI) = (1.2, 2.3); for men RR = 1.4, CI = (1.1, 1.7)). However, the low serum cholesterol-mortality relationship was modified by time, age, and among older persons, activity level. The low serum cholesterol-mortality association was strongest in the first 10 years of followup. Moreover, this relationship occurred primarily among older persons (RR for low serum cholesterol for women 35-59 = 1.0 (0.6, 1.8), for women 70-74, RR = 2.1 (1.2, 3.7); RR for low serum cholesterol for men 35-59 = 1.2 (0.8, 2.0), for men 70-74, RR = 1.9 (1.3, 2.7)). Among older persons, however, the low serum cholesterol-mortality association was confined only to those with low activity at baseline. Factors related to underlying health status, rather than a mortality-enhancing effect of low cholesterol, likely accounts for the excess risk of death among persons with low cholesterol. The observed low cholesterol-mortality association therefore should not discourage public health programs directed at lowering serum cholesterol. (Harris, Feldman, Kleinman, Ettinger, Makuc, Schatzkin. J Clin Epidemiol, 1992;45:596-601.)

- The relationship between cholesterol and 14-year incidence of coronary heart disease was compared for men and women of two age groups, 25 to 64 years and 65 to 74 years. While cholesterol levels of 6.2 mmol/L or higher were associated with a risk of coronary heart disease in the younger group, this was not true for either men or women aged 65 to 74. Further analyses for older persons showed that weight loss modified the cholesterol-heart disease relationship. Those with stable weight showed a positive relationship between cholesterol and coronary heart disease, similar to the younger age group (relative risk [RR] = 1.8 [95% confidence interval: 1.1, 2.9] for men; RR = 1.6 [.7, 3.4] for women). Among those with a weight loss of 10% or more, the relationship of cholesterol to heart disease was inverse (RR = .8 [.5, 1.2] for men; RR = .6 [.3, 1.0] for women). These data suggest that the relationship of cholesterol to coronary disease in healthier older persons may be similar to that in younger person, and that health status should be considered in analyses of cholesterol risk in old age. (Harris, Kleinman, Gillum, Feldman. Ann Epidemiol, 1992;2:35-41.)

PROJECT NUMBER Z01 AG 07060 01 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

March 9, 1992 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Mediators of the Disease-Weight Loss Relation Among Older Persons in the Framingham Heart Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S. Chief, Geriatric Epidemiology Office, EDBP, NIA

Ronald Prior, Ph.D., Scientific Program Officer, Human Nutrition Research Center on Aging

Irwin Rosenberg, M.D., Director, Human Nutrition Research Center on Aging at Tufts University

COOPERATING UNITS (if any)

USDA-Agricultural Research Service

LAB/BRANCH

Geriatric Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.08	

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects — X (b) Human tissues — O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to fund the drawing, transporting, processing and analysis of blood specimens from the Framingham Heart Study cohort in Examination Cycle 22 and the collection of bioelectric impedance data from members of the cohort in Examination Cycle 25.

Biometry Office

The Biometry Office conducts research in the mathematical, statistical and numerical aspects of aging and health. The Office is responsible for methodologies used in research programs related to aging and studies factors which affect health, disease and longevity. The Office performs a variety of functions involving the development and application of mathematical and statistical methods for EDB Program data on the epidemiology and demography of aging and health. The Office also provides statistical consulting, computing, graphics, and data management services to the other units within EDB as well as other Programs within the NIA, other NIH Institutes, other Government agencies, and the private sector.

There have been no changes in the composition of the personnel of the Office during fiscal year 1992; and Dr. Dwight Brock continues as Office Chief. Recruitment of a mathematical statistician to fill a position which has been vacant for 2 years has continued to be a challange. Five candidates have been interviewed and have presented research seminars to the staff; however, the recruitment cannot be completed until the end of the recent hiring freeze. One other personnel matter, however, is much more positive. Mr. Daniel Foley has returned to the Program full time, having completed a period of advanced training in epidemiology and biostatistics at The Johns Hopkins University School of Hygiene and Public Health. This training has been very helpful in providing Mr. Foley additional expertise in conducting research in biomedical, social and other factors related to aging processes, disability, disease and use of health services.

The developmental activities and achievements are summarized below by project.

1. Established Populations for Epidemiologic Studies of the Elderly (EPESE)

As Project Officer for the Yale Health and Aging Project, Mr. Foley continues to oversee the contract requirements for this study. During the past year, staff at Yale University have begun to link the cohort data to Medicare Part A data (hospital utilization) for the period 1985 to 1990. The augmented database will provide greater opportunity for investigation of selected morbidities using the listing of hospital diagnoses from the Medicare data files. In addition, the cohort data are being linked to the National Death Index for ongoing surveillance of vital status and to the state of Connecticut Nursing Home Registry for augmentation of existing nursing home admission data and for continued surveillance of this endpoint.

Ms. Mary Lafferty continues to serve as Project Officer for the East Boston Senior Health Project. In her capacity as data manager for the EDB Program, she has concentrated efforts on the supervision of data management, editing and cleaning at the East Boston site in conjunction with her duties of project monitoring. As a result many improvements have been made in the quality of the East Boston data.

Dr. Brock continues as Project Officer for the Piedmont Health Survey of the Elderly located at Duke University Medical Center. The Duke site is still in the data collection phase, having established its cohort some 4 years later than the other three EPESE sites. In the past year the fourth telephone follow-up survey has been completed and the third in-person survey has begun, following a 4-month delay in receiving questionnaire and protocol clearance from the Office of Management and Budget (OMB). Because of the delay in obtaining clearance, it will be impossible to complete the majority of the interviews and examinations within the time window (within 2 weeks of the anniversary date of the baseline survey interview) originally set for spacing of the follow-up interviews. As a result, many, if not most, of the analyses of data from the third in-person contact will have to be adjusted for unequal length of follow-up, thus requiring use of more complicated analytic techniques than was originally planned and potentially leading to more cautionary statements regarding the conclusions that might be drawn from the findings.

Progress on the third in-person contact at Duke is quite satisfactory, given the limitations described above, with interview response rates of approximately 97 percent and response to the blood draw of about 76 percent. In addition, protocols were added to the scope of the Duke study to include three measures of immune function to be assayed from the blood samples drawn during the current wave of the study. A contract modification was necessary to put the immune function testing into place. This, too, took more time to execute than anticipated, since a full justification for other than full and open competition (JOFOC) was required by the NIH Contracts Office, even though it was believed that sufficient language had been written into the original Duke renewal contract to cover this work. Nevertheless, the project is now in place and work has begun on studies of intraindividual variability and general testing of markers of inflammation and coagulation, including Interleukin 6, XDP (fibrin D-dimers) and Agarose Gel Electrophoresis (AGE).

As in the other EPESE sites, surveillance data on mortality, nursing home admissions and hospitalizations will continue to be collected as the interviews and examinations proceed, and utilization and vital status files will be maintained and submitted with other data provided by the contractor. Also, as in the other sites, Duke will link its cohort to Medicare Part A hospitalization files and to National Death Index files for a period of 5 years following the end of direct contact with the cohort.

One other problem needs to be mentioned with regard to the monitoring of the fieldwork at the Duke EPESE site. Because of the severe restrictions on travel during fiscal year 1992, it has been impossible for the Project Officer to make a site visit. This is a particularly critical problem during the early phases of the third in-person interview and examination, since all problems encountered by the contractor in the field have had to be discussed by telephone. In the past, the opportunity to observe field data collection first-hand has been valuable in noting problems and effecting solutions early in the field effort. At this time it is too soon in the proceedings to know to what extent such problems may have an effect on the overall quality of the data except for the unequal spacing of the observations as cited above.

Ms. Caroline Phillips has served as Alternate Project Officer for the data linkage between Medicare Part A data files from the Health Care Financing Administration (HCFA) and the

original three EPESE sites (East Boston, New Haven and Iowa). During Mr. Foley's training period this year, Ms. Phillips has taken over the liaison activities, arranging meetings with HCFA representatives and conference calls with the appropriate data management personnel at the three sites. Following a preliminary meeting with HCFA personnel to discuss data acquisition strategy, confidentiality requirements and specifications for reimbursement through an interagency agreement, Ms. Phillips coordinated the submission of EPESE cohort identification data to HCFA for matching to the Medicare hospitalization databases for 1985 to 1990. Ms. Phillips coordinated the distribution of the data tapes at the completion of the match and led discussions with the EPESE site data managers concerning strategy and specifications for completion of a pilot match of 500 EPESE participants from each site. Results of the pilot match were reviewed by EDB data management staff and reported at the EPESE Principal Investigators' meeting in April 1992 along with principal and secondary diagnoses from the Iowa site. Further discussions were held with site representatives in June 1992 to specify requirements and schedule for the final HCFA match for all eligible EPESE participants to be submitted to NIA in December 1992. The results of these matches will be extremely useful in defining outcome measures for studies based on risk factor information obtained in the 7 years of interviewing the EPESE participants.

Collaboration and research continues in the area of licensing older drivers. The University of Iowa will do a follow-up study of driving in this cohort through a grant funded in part by the U.S. Department of Transportation (DOT). The purpose of the follow-up study will be to learn more about the process by which older persons lose their privilege to drive in a state that carries a policy for graduated (restricted) driver licensing. Mr. Foley has played an integral part in the development of the follow-up survey and the objectives of the study with investigators from both DOT and the University of Iowa.

In another project on driving behavior, Ms. Phillips collaborated with Dr. David Shinar, a guest researcher at the DOT National Highway Traffic Safety Administration (NHTSA), on studies of older drivers using EPESE Follow-up VI data from Iowa. A paper on vision and elderly drivers by Shinar, Phillips, R.B. Wallace and P.L. Colsher (of the Iowa site) was presented at a workshop on driver vision standards and testing in Toronto in October 1991. The analysis of driver record and accident data is continuing.

Ms. Phillips and Ms. Lafferty were coauthors with Dr. Eleanor Simonsick on a paper comparing mortality, morbidity and disability after 3 years and 6 years of follow-up of physically capable older adults with non-active, moderately active, and highly active levels of physical exercise. A final version of the paper has been completed and submitted for publication.

Mr. Foley collaborated on a paper discussing a multi-site study of the prevalence and correlates of prescription and over-the-counter medication use in the EPESE which was revised and accepted for publication in the Journal of Gerontology. In summary, analgesic and cardiovascular drugs were the most prevalent drugs used by men and women at all sites (35 percent of respondents), and no significant associations were found consistently across sites in socioeconomic factors which relate to prescription or over-the-counter drug use.

Gastrointestinal agents, nutritional supplements, central nervous system agents, and endocrine/metabolic agents were each reported by at least 9 percent of respondents at each site. Health status measures, including depression, physical functioning, and use of a hospital in the prior year at each of the sites, were consistently associated with taking medications. Although much research is devoted to polypharmacy in the elderly, studies of under-use of medications are not common. Results showed that between 10 and 29 percent of those reporting their health as fair or poor also reported taking no prescription medications. This finding suggests that there are pharmacologic needs that are not being met for some individuals.

Mr. Foley has been participating in another area of analysis in collaboration with Dr. Marcel Salive and investigators from Duke University considering the issue of predicting nursing home admission in a biracial population based on data from the Piedmont Health Survey of the Elderly. The findings were submitted to the American Journal of Public Health and showed that the age-adjusted 3-year incidence of nursing home admission was 8.5 percent among whites and 6.4 percent among African-Americans. After controlling for vital status and other health and socioeconomic characteristics, African-Americans still were one-half as likely as others to be institutionalized over the 3- year period of follow-up. We conclude that racial differences in nursing home utilization cannot be explained solely by differences in the measures of financial access and social support, but rather a clearer understanding of underlying historical and cultural trends may be necessary to explain the difference.

Mr. Foley is continuing his collaboration in the area of nursing home admissions with investigators from both Duke University and Yale University, focusing on the relationship between extended family networks, social support, and utilization in these two EPESE sites. Preliminary findings are showing a striking difference between men and women in the type and mix of family networks which reduce the risk for admission. The presence of a spouse is the critical element for men, whereas for women, the presence of child or a close relative reduces the risk.

A considerable amount of effort has gone into the continuing analysis of EPESE data on change in physical function. Drs. Jack Guralnik and Dwight Brock have been part of a large collaborative team working on several fronts to complete the first analysis of these data, using some newly-developed and recently-modified analytic techniques involving both random effects linear models and Markov models. Preliminary results from the random effects models indicate that for the Katz, Rosow-Breslau and Nagi physical function scales at three of the EPESE sites, mean function scores decline cross-sectionally both as a linear and a quadratic function of age, and that this decline is exacerbated across time. That is, older individuals appear to show steeper declines at later interview times in the EPESE follow-up data. On the other hand, results of the Markov models, which do not model function scores, but the presence or absence of impairment in functioning at each time point in the follow-up, show that there were improvements by some individuals in functioning (i.e., recovery from impairment) although the overall trend appears to be in the direction of decline. These results are being prepared for presentation at the November meeting of the American Public Health Association.

Ms. Katalin Losonczy has continued her collaboration with Drs. Lon White, Marcel Salive, and Robert Katzman on the analysis of EPESE longitudinal data on change in mental status and cognitive function over the three time periods represented by EPESE in-person interviews at the first three sites. Results of the analysis have shown an effect for education in the levels of Mental Status Questionnaire scores in these data. There remain to be resolved some issues regarding controlling for site differences in the analysis. A final draft of the paper has been prepared for review by the collaborators to be submitted for publication.

Ms. Losonczy is conducting a collaborative analysis (with Drs. Tamara Harris, Eleanor Simonsick, Joan Cornoni-Huntley and others) of EPESE data seeking to determine the effects of baseline measures of body mass index on subsequent mortality. Data from all four sites are being utilized in this work, thus necessitating some adjustments in methodologic approaches to be taken in the analysis. Data from the first three sites extend to 6 years of follow-up, whereas Duke data extend only to 3 years. Using the middle quintile of body mass index (weight in kilogram divided by the square of height in meters) as the reference group, the collaborators have found that persons age 70 and older whose body mass index falls in the first quintile (i.e., lowest body mass index group) appear to be at greater risk of mortality while those whose index is greater than the reference group are at somewhat less risk. Numerous factors ascertained at EPESE interviews are being controlled in the analysis. This project will be continuing.

Dr. Brock is continuing analytic activity on several papers currently underway. He collaborated with Drs. Gerda Fillenbaum, Elizabeth Corder and Joe Hanlon of Duke University on a paper entitled "Concomitants of prescription and over-the-counter drug use in black and white community resident elderly." A preliminary version of this paper was presented at the Gerontological Society meeting in November 1991, and a revised version was submitted to the American Journal of Public Health. The study indicates the relative unimportance of demographic characteristics and substantial importance of need and enabling characteristics in explaining prescription drug use, and points to difficulties in explaining over-the-counter drug use.

Dr. Brock and Dr. Jon Lemke of the University of Iowa have continued work on an analysis of baseline data on hearing loss from all four EPESE sites. Preliminary models have shown an association with the use of tricyclic antidepressant drugs and with loop diuretics in the Iowa and East Boston sites. The data from New Haven and North Carolina are currently being prepared for analysis, using a software package that accounts for the complex sampling designs in those two locations. It appears that failure to test for interactions that involve the use of both drugs results in a misleading conclusion about the effects of the two drugs; that is, if the drugs are examined separately, no effects appear to be statistically significant, whereas, if both drugs are included in the models, the effects attain significance in the presence of the interaction term. Further exploration of these data will continue as the data from the other two sites are brought into the analysis.

Finally, Dr. Brock has collaborated with Drs. Lisa Berkman, Robert Glynn, Ben Amick and Frank Kohout on an analysis of data relating social class at baseline in the EPESE to

subsequent mortality. Some methodological issues remain to be resolved before final analysis is completed on this project. Also, Dr. Brock has collaborated with Drs. Glynn and Havlik on analysis of change in blood pressure using the three sets of blood pressure measurements from the first three sites. A variety of modeling and substantive issues are being discussed in this analysis.

2. National Health and Nutrition Examination Survey (NHANES) and NHANES Epidemiologic Follow-up Survey (NHEFS)

Biometry Office staff continue to conduct and complete analyses of data from the various phases of the NHEFS, while the EDB Program as a whole continues its support of the newer survey known as the NHANES III. Ms. Losonczy has worked with Drs. Wayne Giles and Steven Kittner at the University of Maryland on analysis of black/white differences in stroke as ascertained in the 1986 and 1987 follow-ups of the NHANES I, or NHEFS. Ms. Losonczy acted as liaison with Tasqe, the EDB computing support contractor in this analysis. This also involved updating an earlier analysis of NHEFS data on racial differences in stroke.

In another area, Ms. Losonczy has completed an analysis of use of vitamin E and age-dependent disease in the NHANES II sample. Here the hypothesis was that use of vitamin E would lead to decreased risk from chronic diseases such as cancer and cardiovascular disease. A draft paper has been completed and is currently under review by the coauthors.

Ms. Lafferty has continued her involvement in a collaboration on a project to make extensive comparisons among the NHEFS, the EPESE and the National Center for Health Statistics Supplement on Aging (SOA). This analysis will compare such variables as weight, age, and blood pressure distributions and provide basic information on commonalities and differences among these three data sets.

3. Survey of the Last Days of Life

Analytic activity has been continuing for this study of the dying process in a community sample of older decedents in Fairfield County, Connecticut. A paper was published on the characteristics of the 260 decedents whose underlying cause of death was recorded as cancer on the death certificate. Details of the findings are given in the Research Highlights section of this report.

An analysis is currently under way on the decedents' use of hospital, institutional and home care in the last 90 days of life. Informant responses to the questionnaire for this study indicate that the decedents spent an average of 37 days in either a hospital or nursing home (including personal care homes) during the last 3 months of life and that hospital days decreased slightly with age, while nursing home days increased dramatically, with women's utilization about 40 percent higher than men's. The responses also addressed the number of transitions among the different health care settings and indicated that while 85 percent of

patients resided in three or fewer places immediately prior to death, almost three-fourths made only one transition to or from a health facility. Regression models were used to assess the relationship between the number of days of patient care residency and a variety of predictors which can be classified into three broad groups -- health status and functioning, social support, and symptoms and disease history, including a number of well-known chronic conditions affecting older persons. Preliminary results showed that the variation in the number of hospital days experienced by these individuals was slight and could not be modeled effectively by the variables measured in the study. Indeed, hospital length of stay is undoubtedly influenced strongly by rules and policies governing Medicare reimbursement. On the other hand, both institutional days and community days were modeled well for these persons. For each outcome for men, strong associations were observed with marital status, incontinence, and a history of Alzheimer's disease, with statistically significant effects for hearing ability, history of stroke, presence of children, and poor physical functioning. For women, the strongest associations were for Alzheimer's disease, presence of children, and poor physical functioning, with weaker effects for marital status and incontinence. For both men and women the effects of age on length of stay in an institution or in the community were no longer significant once the other variables were accounted for in the models. Additional results from this study are being prepared for presentation at the American Statistical Association meeting in August.

The analysis of data on social isolation at the time of death (with Dr. Toni Miles of the University of Illinois) is continuing. Whereas a number of potential risk factors were identified previously, additional analyses are being done to examine a sizable portion of the sample of the decedents who did not see any relatives during the 3 days prior to their deaths. A report of fair or poor health immediately before death and indications of awareness of impending death by the decedent appear to be more likely for those individuals who did not see relatives 3 days before they died. Further, previous results which showed a methodological effect of the use of the telephone in the interview with the informant did not hold up as additional factors were included in the analysis. We anticipate completion of this analysis by the end of fiscal year 1992 and the submission of an abstract of a paper to be presented at the 1993 American Statistical Association Winter Conference in January of 1993.

Graphical work has continued on an analysis of data on changes in mental and physical functioning in the last year of life, being conducted by Ms. Losonczy in collaboration with Drs. White and Brock. Upon completion of this work, statistical models will be fitted to examine associations between an index of functioning and a variety of predictor variables available from the data set. This work will be continuing.

4. National Mortality Followback Survey

An interagency agreement was signed with the National Center for Health Statistics (NCHS) to support the oversampling of centenarian decedents in the 1993 National Mortality Followback Survey (NMFS). The purpose of the study will be to obtain a variety of information about the decedents' health status, use of health care services in the last year of

life, lifestyle factors and other characteristics immediately prior to death in a large nationally representative sample. It is hypothesized that centenarians make less use of health care services than persons who die at younger ages, and that costs of care are lower both because of fewer days spent in a facility and because of lower costs per day. A second hypothesis is that a compression of morbidity takes place among persons who die at extreme old age: that is, for years prior to death not associated with a terminal illness the use and costs of service are less for centenarian decedents than for younger decedents. Thirdly, lifestyle factors, including increased physical activity/vitality and favorable health habits contribute to increased longevity in these older decedents.

Planning work has been under way this year with staff of the NCHS. Drs. Simonsick and Brock have been working closely with NCHS personnel on the development of specific questionnaire items to be included in the survey, especially in the areas of physical and cognitive functioning, measures of vitality and use of services in the last year of life. After several revisions and testing by the NCHS cognitive science laboratory, a draft questionnaire has been completed and submitted to the Office of Management and Budget for clearance for a formal pretest of all questions, measurement issues and field procedures to be conducted during the fall of 1992.

Actual sampling of the universe of 1993 death certificates is expected to begin with deaths registered in the January 1993 NCHS Current Mortality Sample (CMS), a 10 percent sample of all deaths recorded in state vital statistics offices and submitted to NCHS each month. One hundred percent of all deaths of centenarians obtained in the CMS will be eligible for the survey. Next-of-kin interviews will be conducted by Census Bureau interviewers approximately 5 months after the deaths are recorded. In addition, data will be available from the death certificate, from facility abstract records and from medical examiner/coroner abstracts. The next-of-kin informants will be interviewed by telephone where possible, with in-person follow-up for those respondents who cannot be reached by phone. Health care facilities will receive mail questionnaires directed to administrators and medical records and billing department personnel.

An attempt will be made to obtain identifying information to facilitate linking of the sample members' survey data to utilization information available from the Medicare files of the Health Care Financing Administration (HCFA). This will greatly facilitate the study of compression of morbidity, especially for the years prior to the terminal illness.

The timetable for the study calls for a pretest to be done in four states in mid-October of 1992. The main survey data collection period will be between June of 1993 and July of 1995. A public use data tape is anticipated to be available in December of 1995.

5. Data Management/Computing

The heart of the data quality management for the EDB Program continues to reside with the statistical computing/programming staff, directed by Ms. Mary Lafferty. The staff continues to analyze data and design programs to check data for completeness, accuracy and

consistency. Ms. Lafferty has prepared drafts of EPESE baseline data, documentation, tables and file formats for archiving the first set of data from these studies. Plans now call for these data to be released by the beginning of fiscal year 1993 in October, pending final governmental approval.

Ms. Phillips' data management emphasis this fiscal year has been on final cleaning and editing of EPESE Follow-up VI interview files and endpoint files consisting of mortality, hospitalization and nursing home use data for the first three EPESE sites (East Boston, Iowa and New Haven). In addition, the baseline interview file for the fourth EPESE site (Duke) has been given final acceptance by the data management staff. Other files are currently under review. The data management staff as a group have worked very closely with the computing support contractor, Tasqe, Inc., to update editing programs and to develop new programs as they are needed for this process.

In other data management activities, Ms. Phillips continues to provide support for the editing and file management for the Survey of the Last Days of Life. A revised data set and documentation were completed during fiscal year 1992 for use by EDBP data analysts. Ms. Lafferty serves on the data management committee for the new Women's Health and Aging Study (WHAS) which is now in the final planning stages for the initial contact with the cohort to be established. This kind of advanced planning for later data needs will make data management work much smoother as the study progresses, and it should facilitate analysis of the data in the future.

A good deal of work was undertaken by the computing staff in preparation for and in carrying out the move from the Federal Building to the Bethesda Gateway Building in October of 1991. Ms. Lafferty and Ms. Phillips organized the placement of computing equipment, backed up all fixed disks, carried out an inventory of the equipment and coordinated packing and disbursement of computer related supplies. In addition, staff provided support for revising the EDB/NIA local area network as a result of the move. In addition, Ms. Phillips serves as de facto network administrator for EDB's local network and is largely responsible for setting up hardware and software on PC's, trouble-shooting and making minor repairs to the equipment.

Upon Ms. Lafferty's recommendation, the Program purchased five new 486 personal computers. One has been set up as statistical workstation with extra memory and disk capacity and a math co-processor. Ms. Lafferty worked with Dr. Luigi Ferrucci, a visiting scientist from Italy, to download and install the EPESE database on this PC for use with PC SAS by EDB data analysts. In addition, Dr. Ferrucci developed a WordPerfect-based system for creating batch files of program commands to submit jobs to PC SAS. It is now possible to run PC SAS on our own data quickly without having to use the NIH mainframe computer. This is one of the goals set for the staff some time ago when the conversion to personal computing began in this Program. This system is also able to process data using the BMDP software package in a timely fashion. As a result, EDB analysts have begun to convert to PC-based data analysis, thus effecting considerable savings for the EDB computing budget.

The computing staff, and particularly Ms. Lafferty, serve as liaison for the computing support services contractor, Tasqe, Inc. The contract has been continued for a second year, with many EDB staff utilizing the services of the contractor on a large variety of projects, from installation and evaluation of new software to management and analysis of data from many sources. One such project involves the development of an automated telephone directory and travel voucher tracking system for the EDB support staff. In addition, a system has been proposed for monitoring EDB procurements within the Program to assist in tracking the Program's operating budget.

Perhaps the most visible and recognizable output of the computing staff is the production of graphics, an activity in which Ms. Josephine Cruz has taken primary responsibility, with Ms. Phillips as backup. In addition to providing computer-generated slides and hard copy graphics for the entire EDB Program staff, Ms. Cruz has completed graphics work for several offices outside the Program, including the Office of Financial Management and Information Systems Branch, the Office of Interdisciplinary Research, the Biology of Aging Program, the Office of Planning, Analysis and Evaluation, the Office of International Activities and for a guest researcher, Dr. Lenore Launer, visiting from The Netherlands.

Ms. Cruz provided consultation on presentation of data for Dr. Ferrucci while he was a visiting scientist with the Program for 7 months. From that work a number of papers will be published. Also, Ms. Cruz provided similar consultation for Dr. Sergio Constanzo, an Italian guest researcher in the Program for 4 months. Finally, Ms. Cruz has provided training in the Zenographics software package to several NIA and EDB personnel.

6. Statistical Methodology

Follow-up work on the evaluation of models for application to EDB longitudinal data sets has continued during this fiscal year. The random effects linear models included in the previous evaluation project have proven to be useful in analyzing data on changes in physical functioning at all four EPESE sites, even though fewer years of follow-up are available at the Duke site. The results of the modeling have shown remarkable consistency in the factors affecting change in function. The results of the model evaluation continue to draw outside requests, primarily from universities, for information about the project. Dr. Brock gave presentations at the University of South Carolina School of Public Health in October of 1991 and at The Johns Hopkins University School of Hygiene and Public Health in April of 1992. In addition, Dr. Laurel Beckett, who led the investigation, has submitted an abstract which was accepted for presentation at the International Biometric Society meeting in New Zealand in December of 1992. Dr. Brock is a co-author of the paper. Also, Dr. Brock has submitted a poster paper on a related subject to be presented at the upcoming NIH conference of biostatistics to be held in Bethesda in January of 1993.

A second project on longitudinal data modeling has made substantial progress during the year as well. Dr. Beckett, working with Dr. Brock and Dr. Paul Scherr of the Centers for Disease Control, has completed a project to extend the Markov models for longitudinal data, also evaluated in the project described above, to the complex sample survey setting. The results of this work will enable analysts to study longitudinal changes in state using the New

Haven and Duke EPESE data, while accounting for the clustering inherent in the data introduced by the complex designs used at those two sites to collect the data. A computer program written in FORTRAN is now available for EDB to use in fitting these models. Further extensions of the Markov models are now being considered, including extensions to modeling mortality as an absorbing state, relaxing the assumption of equally-spaced data to model random sojourn times in the various states of the system, and the introduction of time-varying covariates, so that one does not have to make the assumption that the covariates remain fixed throughout the period of observation of the phenomenon under study.

The EDB Program has received a test copy of a software program to fit Grade-of-membership models to EDB longitudinal data. This was one of the models we attempted to evaluate along with the random effects and Markov models with the physical function data. However, at the time of the evaluation the software permitted analysis only of the test data provided by the developer of the software. The revised program is believed to be corrected so that other data sets can be analyzed with it. Over the course of the next several months, we plan to evaluate the revised program using our EPESE data.

The Research Triangle Institute, in a contract with the National Cancer Institute, has produced software for fitting proportional hazards regression models to continuous data from a complex sample survey. This is continuation work on the project which resulted in the SUDAAN package, supported in part by an interagency agreement between EDB and the NCHS, reported in previous Annual Reports. The new software, now in a test phase, will also enhance analytic capability for data from such studies as the New Haven and Duke EPESE as well as the NHANES Follow-up Study and the Longitudinal Study on Aging (LSOA).

Dr. Brock is working with Dr. Jon Lemke of the University of Iowa on the testing of interaction effects in logistic regression analyses when the data are subject to order restrictions. The initial application will be to the hearing loss data described earlier in this report. In essence, when two predictor variables are operating in a synergistic manner in a given data set, the testing of either or both of them as predictors in the absence of an appropriate interaction term tends to show a dampening of the effects of either of them on the outcome of interest. This phenomenon is being thoroughly investigated.

7. Consultation, Collaboration and Other Professional Activities

Biometry Office staff have served in a variety of consultative, collaborative and review capacities.

Dr. Brock served as a reviewer for the NCI Prostate, Lung, Colon, and Ovarian (PLCO) cancer screening clinical trial, a multi-year, multi-million dollar project to test the effectiveness of screening for those specific cancers. This review process has been carried out over a period of several months and involves the evaluation of a large number of proposals from various cancer centers around the country.

Mr. Foley has served as a reviewer for the Journal of Aging and Health, and Ms. Losonczy serves as the EDB representative to the NIA nutrition advisory committee.

Ms. Losonczy served as a member of an advisory committee for a research project sponsored by the American Water Works Association Research Foundation in Denver, Colorado. This required comments following her review and ranking of five proposals for studies on the relationship between aluminum in drinking water and Alzheimer's disease. These review activities will be continuing.

Ms. Losonczy attended a workshop on antioxidants in the prevention of human atherosclerosis, sponsored by the National Heart, Lung, and Blood Institute, in September of 1991, a conference on nutrition and aging in Little Rock, Arkansas, also in September, and the Gerontological Society of America meeting in San Francisco in November of 1991.

Dr. Brock continues as the Council of Chapters Representative to the American Statistical Association from the Washington Chapter. He also continues to serve as a member of the Washington Statistical Society Quantitative Literacy Group, which fosters the teaching of statistics in elementary and secondary schools in the Washington area.

8. Awards

Caroline Phillips was our awardee this year for the Secretary's Employee-of-the-Month Award for the extra work she performed on the data processing/office automation system this fall in association with our move from the Federal to the Gateway Building.

Dwight Brock received an award from the Washington Statistical Society (WSS) in appreciation of service to the society in the initiation and establishment of the WSS Quantitative Literacy Group and for serving as co-chair during the committee's first 2 years.

Research Highlights for FY92

- Beginning in 1982, the 3-year incidence of nursing home admission was determined for community-dwelling residents aged 65 and over in three EPESE communities (East Boston, MA (4%); New Haven, CT (9%); and Iowa and Washington Counties, IA (12%)). A common methodology was used to collect baseline risk factor and follow-up data on nursing home admissions among persons in each community as part of the EPESE. A multivariate logistic regression model of baseline risk factors that included the participant's age, race, sex, history of prior admission, ADL limitations, cognitive function, living arrangements, and level of income predicted 80% of the users in each community. (Foley, Ostfeld, Branch, Wallace, McGloin, Cornoni-Huntley. J Aging Health, 1992;4:155-73.)

- Cancer has become one of the chronic diseases requiring special adaptation in regard to the life style of the cancer patient and the quality of life. The assessment of quality of life in the cancer patient has been evaluated through questionnaires to relatives and friends as well as through the social experience of the patients. Of particular interest is the quality of life of older patients in the last days of life. In the NIA Survey of the Last Days of Life, for which 260 respondents reported cancer as the underlying cause of death, approximately 1/5 of the decedents were reported to have been in moderate or severe pain 6 hours before death, with little change from the previous month. Some 35% of the decedents showed awareness of impending death, either through behavior or from physician information. Cancer patients in this study carried a 2.5-fold risk of poor physical functioning within the year prior to death relative to the month and day before death, and a 4-fold increased risk of poor functioning more than one year before death relative to the month and day before death. (Kaiser and Brock. In Vivo, 1992;6(4):333-37.)

- General characteristics of older decedents in the NIA Survey of the Last Days of Life are described in four broad categories of substantive interest: Lifetime History of Selected Diseases and Conditions, Circumstances Surrounding Death, Transitions Between Residential and Health Care Settings in the Last Three Months of Life, and Trends in Health and Functional Status in the Last Year of Life. Selected findings include an estimated lifetime history of 11.2% of the decedents with a diagnosis of Alzheimer's disease, 11.0% with a hip fracture, and 35% ever admitted to a nursing home. Some 53% were reported to have died in their sleep, 35% showed some awareness of impending death, and 89% saw various members of their families in the last three days of life. Of the 45% who spent the night before they died in a hospital, some 80% had been staying in a private residence before being hospitalized, whereas, of the 25% in a long-term care facility immediately before death, 42% had previously been in a hospital and 48% in a private residence. Trends in health status showed a steady decline from 53% in good or excellent health the year before death to 11% on the day before death, and while 59% were reported to be freely mobile a year before death, only 13% had no limitation in mobility on the day before death. On the other hand, a majority of decedents (51%) were reported to have maintained good cognitive

functioning on the day before death, having declined from 87% for the year before death. (Brock, Holmes MB, Foley, Holmes D. The Epidemiologic Study of the Elderly, Wallace RB, Woolson R, eds. New York: Oxford University Press, 1992;315-32.)

CONTRACT

Name and Number: Yale University (N01-AG-0-2105)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $339,216

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1989-93) will be based on the use of the National Death Index for mortality and the Health Care Financing Administration's Medicare data for morbidity.

Major Findings: Health consequences of living with a cognitively impaired spouse were identified in a sample of 318 spouse pairs, drawn from a representative sample of noninstitutionalized elderly individuals in the EPESE New Haven community. Principal findings were that (a) cognitive impairment in wives was significantly ($p < .05$) associated with elevated systolic blood pressure in husbands, whereas cognitive impairment in husbands was not associated with systolic blood pressure in wives; (b) cognitive impairment in wives was significantly ($p < .05$) associated with perceived declines in health status in husbands, but cognitive impairment in husbands was not related to perceived declines in health status in wives; (c) the relationships between wives' cognitive impairment and husbands' blood pressure and perceived health status were not mediated through limitations in activities of daily living in wives, chronic conditions in wives, or perceived inadequacy of emotional or instrumental support; and (d) cognitive functioning in one spouse was not related to health behaviors in the partner. Taken together, our results suggest that spouses of community-dwelling impaired individuals are not so severely affected that they cannot function normally. However, they suffer from health problems that can have serious consequences (ref. 1).

The effect of characteristics of social networks and support on depressive symptoms in the elderly were examined in 1,962 noninstitutionalized persons 65 years and older from the New Haven EPESE in 1982, who were available to give a complete follow-up interview in 1985. Baseline depression, functional disability in 1982, and any change in disability by 1985 were considered as additional influences on 1985 depression, requiring adjustment along with sociodemographic variables. Multiple regression procedures were used to simultaneously examine the variables. Baseline depression, functional disability, and change in functional disability made the largest contribution to explaining the variance in depression. Among the social support and network characteristics, loss of a spouse, adequacy of emotional support, and its change during 1982-1985 made the largest contributions. Other significant characteristics in relative order of magnitude of effect, based on contrast tests, included tangible support adequacy and its change, loss of a confidant between 1982 and 1985, number of children making weekly visits and change in this number by 1985, and the absence of a confidant in both 1982 and 1985. For mental health outcomes, these findings emphasize the need to consider specific dimensions of social support and networks rather than global measures (ref. 2).

Publications

1. Moritz DJ, Stanislav K, Ostfeld AM. The health impact of living with a cognitively impaired elderly spouse: Blood pressure, self-rated health, and health behaviors. J Aging Health 1992;4:244-67.

2. Oxman TE, Berkman LF, Kasl S, Freeman DH Jr., Barrett J. Social support and depressive symptoms in the elderly. Am J Epidemiol 1992;135:356-68.

CONTRACT

Name and Number: Peter Bent Brigham Hospital (NO1-AG-0-2107)

Title: Established Populations for the Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $349,380

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1983-93) will be based on the use of the National Death Index for mortality and the Health Care Financing Administration's Medicare data for morbidity.

Major Findings: In hematologic tests obtained from 3,946 adults aged $\geq$71 years in three EPESE communities (East Boston, MA; Iowa and Washington counties, IA; and New Haven, CT), it was concluded that age was significantly associated with both hemoglobin levels and anemia, with a stronger effect in men compared with women, even after simultaneously adjusting for demographic characteristics and health status. The decline of hemoglobin and concomitant increased anemia with age was not necessarily a result of "normal aging" so the detection of anemia in an older person should prompt appropriate clinical attention. Hemoglobin level was inversely associated with age, although this was more pronounced in men than in women. The proportion anemic was equal for men and women aged 71-74 years (8.6%) and increased differentially with age, reaching 41% and 21% for men and women aged $\geq$90 years, respectively. Hemoglobin and anemia were independently associated with age, race, body-mass index, smoking, cancer, hospitalization, renal insufficiency, and hypoalbuminemia. The adjusted relative odds of anemia for a 5-year increase in age was 1.5 (95% [CI] 1.3-1.8) for men and 1.2 (95% CI 1.1-1.4) for women (ref. 1).

We analyzed data from 4,115 persons aged 71 years and older who had blood drawn at a home visit in three EPESE communities to examine the cross-sectional distribution of serum albumin and correlates of hypoalbuminemia. Mean albumin was lower among older persons, from 41.6 g/l in men aged 71-74 years to 38.5 g/l in men 90 years or older, and from 41.1 g/l to 38.9 g/l in women of the same ages, respectively. Hypoalbuminemia (albumin <35 g/l) was observed in 3.1% of subjects. Hypoalbuminemia and lower serum albumin were independently associated with anemia, recent diagnosis of cancer, two or more limitations in activities of daily living, residence in a nursing home, heavy cigarette smoking (>1 pack/day), and older age. A 10-year age increment was associated with 0.8 g/l lower serum albumin and odds ratio of 1.56 (95% CI 1.14, 2.13) for hypoalbuminemia after adjusting for demographic factors and health status. Characteristics associated with serum albumin may confound the reported relationship between serum albumin and mortality (ref. 2).

Data from three EPESE populations suggest that the relationship of low to moderate alcohol consumption with reduced total and cardiovascular mortality, which are well documented in middle age, also occur in older populations. Subjects with prior myocardia infarction, stroke, or cancer, as well as those lacking alcohol consumption data, were excluded from statistical analyses leaving 2,694 subjects in East Boston, MA, 2,293 subjects in Iowa, and 1,904 subjects in New Haven, CT. Main outcome measurements were alcohol consumption, total mortality, cardiovascular mortality, and cancer mortality. Low to moderate alcohol consumption was associated with statistically significant lowered total as well as cardiovascular mortality in East Boston and New Haven. The relative risks of total mortality for low to moderate consumers of alcohol compared to those consuming no alcohol in the previous year were 0.7 (95% CI 0.6-0.8) in East Boston and 0.6 (95% CI in New Haven. For cardiovascular mortality, the RRs were 0.6 in East Boston and 0.5 in New Haven (95% CI's exclude null). These results persisted after control for potential confounding variables. In Iowa, there were no significant differences in total cardiovascular mortality according to alcohol consumption patterns. For cancer mortality, there were no significant associations with alcohol consumption in any of the three populations (ref. 3).

Publications

1. Salive ME, Cornoni-Huntley J, Guralnik JM, Phillips CL, Wallace RB, Ostfeld AM, Cohen HJ. Anemia and hemoglobin levels in older persons: Relationship with age, gender, and health status. J Am Geriatr Soc 1992;40:489-96.

2. Salive ME, Cornoni-Huntley J, Phillips CL, Guralnik JM, Cohen HJ, Ostfeld AM, Wallace RB. Serum albumin in older persons: Relationship with age and health status. J Clin Epidemiol 1992;45:213-21.

3. Scherr PA, LaCroix AZ, Wallace RB, Berkman L, Curb JD, Cornoni-Huntley J, Evans DA, Hennekens CH. Light to moderate alcohol consumption and mortality in the elderly. J Am Geriatr Soc 1992;40:651-57.

CONTRACT

Name and Number: Duke University Medical Center (N01-AG-1-2102)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: January 1, 1991

Current Annual Level: $1,888,054

Objectives: The purpose of this project is to conduct epidemiologic investigations in a biracial elderly population, 65 years of age and older, selected from both urban and rural locations.

Methods Employed: Descriptive and analytical epidemiologic studies of existing problems and surveillance of newly developing problems all with an emphasis upon future intervention and prevention have been conducted. Investigators conducted cross-sectional and prospective studies as well as more detailed problem-related studies in a carefully defined and accessible population using standard field and analytical techniques.

Significance to Biomedical Research: The NIA began funding three population studies of the elderly to determine the influences of social, environmental, behavioral, and economic forces on the mortality, morbidity, and utilization of health services in the elderly. These studies, however, were not fully representative of the U.S. elderly; specifically, they did not include a significant proportion of blacks. It is well known that distributions of certain risk factors and diseases differ between U.S. blacks and other racial groups. Therefore, the purpose of the 1984 contract was to conduct epidemiologic investigations in an elderly population of which at least 50 percent is black in order to develop new knowledge concerning the medical and social factors in health and diseases of the aging black population. In addition, both black and white subgroups in the study exhibit an excellent distribution on indicators of socioeconomic status, and participants have been selected from both urban and rural locations.

Proposed Course: A new 7-year contract was awarded to Duke University Medical Center January 1, 1991, to conduct a third in-person survey wave and continue surveillance for major endpoints. In this wave, begun in May 1992, Duke is gathering information comparable to that obtained by the other EPESE sites as well as items which are important for the study of the health of older black persons and racial and urban/rural differences. Physical performance measures being taken include tests of balance, a timed walk, chair stands, a test of shoulder range of motion, and functional reach. Waist/hip ratio, height and weight, and vision are also being ascertained. Blood assays include complete blood count, automated serum chemistries, and HDL cholesterol. A supplement to the 1991 contract was added in fiscal year 1992 to obtain additional assays of blood samples for markers of immune function, including interleukin 6, XDP (fibrin D-dimers) and Agarose Gel Electrophoresis (AGE). Items repeated in the previous two waves which have significance for epidemiologic exploration and explaining black/white differences are also included, since observations at

three points in time will permit the kind of causal modeling for which the EPESE data are ideally suited.

Major Findings: Data from the Duke EPESE suggest that there are distinct racial differences in medication-use patterns among the elderly. Drug-use patterns among black and nonblack community-dwelling elderly were compared in a stratified probability household sample of 4,164 community residents aged 65 or older from this population (65 percent women, 54 percent black, mean age 73.56$\pm$6.74y). Data were weighted to present the population in this 5-county urban and rural region in Piedmont, NC. Fewer blacks reported the use of over-the-counter (OTC) medications and total medications than did nonblacks (66 vs. 76 percent and 88 vs. 92 percent, respectively; $p > 0.001$). Compared with nonblacks, blacks reported using a lower mean number of prescription (2.02 vs. 2.35; $p > 0.001$), OTC (1.12 vs. 1.42; $p > 0.001$), and total (3.14 vs. 3.77; $p < 0.001$) drugs. The therapeutic medication categories varied by race for prescription cardiovascular, analgesic, and central nervous system (CNS) drugs and OTC nutritional supplements. More nonblacks than blacks reported the use of analgesics (62.5 vs. 55.6 percent, respectively; $p > 0.001$), CNS drugs (26.1 vs. 14.2 percent, respectively; $p > 0.001$), nutritional supplements (27.5 vs. 16.9 percent, respectively; $p > 0.001$), and gastrointestinal agents (29.0 vs. 23.5 percent, respectively; $p > 0.001$). Blacks were more likely to report problems in managing their medications than were nonblacks (9.0 vs. 6.1 percent, respectively; $p \leq 0.001$) (ref. 1).

Advanced age among the elderly has been hypothesized to be a risk factor for depression, yet extant data do not uniformly support this hypothesis. The paucity of sufficiently large and representative samples of both the young-old and old-old and the failure to control for critical variables known to confound the association between advanced age and depression have prevented testing this hypothesis. The Duke EPESE assessed 3,998 community-dwelling elders (65+) for depressive symptoms using a modified version of the CES-D and relevant control variables. Depressive symptoms were associated in bivariate analysis with increased age, being female, lower income, physical disability, cognitive impairment, and social support. In a multiple regression analysis, the association of age and depressive symptoms reversed when the above confounding variables were simultaneously controlled. The oldest old suffered fewer depressive symptoms when factors associated with both increased age and depressive symptoms were taken into account. Because many of these factors can be prevented (such as decreased income, physical disability, and social support), the uncontrolled association between age and depressive symptoms can potentially be modified (ref. 2).

Publications

1. Hanlon JT, Fillenbaum GG, Burchett B, Wall WE, Jr., Service C, Blazer DG, George KL. Drug-use patterns among black and nonblack community-dwelling elderly. Ann Pharmacother 1992;26: 679-85.

2. Blazer D, Burchett B, Service C, George LK. The association of age and depression among the elderly: An epidemiologic exploration. J Gerontol 1991;46:M210-15.

PROJECT NUMBER Z01 AG 07030 04 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NHANES III: Health of Older Person (Baseline Survey)

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Richard J. Havlik, M.D., M.P.H., Associate Director, EDBP NIA
Kathy Lonsonczy, M.S., Biometry Office, EDBP, NIA
Robert Murphy, Division of Health Examination Statistics, NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.05	

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects O (b) Human tissues O (c) Neither

O (a1) Minors

X (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NHANES III is a multi-agency collaborative survey designed to estimate the prevalence of diseases and risk factors in some 30,000 Americans and conducted by the National Center for Health Statistics. Special efforts are being directed to collection of data from interviews and examinations for the population over age 60 and the oldest-old.

Phase I of NHANES III, a national sample in itself and about one-half of the projected total, is completed. The overall examination rate is 78.36.7 percent and this rate is higher than for NHANES II. However, the response rates are somewhat lower for those 75 years and older. Home examinations are being done to improve the participation rate in older persons.

Biomedical examination content of particular interest to EDB Program investigators includes 24-hour dietary recalls, bioelectrical impedence for body composition, bone density measurement, knee x-rays, and an assessment of physical functioning. Phase I data files are becoming available for preliminary use. Phase II of NHANES III data collection is continuing at the same time and early participation rates have been high..

PROJECT NUMBER Z01 AG 07050 01 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1993 National Mortality Followback Survey

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Dwight B. Brock, Ph.D., Chief, Biometry Office, EDBP, NIA
Eleanor Simonsick, Ph.D., Epidemiology and Demography Office, EDBP, NIA
Paul Placek, Ph.D., Chief, Followback Survey, NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.08	

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects O (b) Human tissues O (c) Neither

O (a1) Minors

X (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to support the collection and analysis of data on cause of death and characteristics of the last year of life in the planning of the 1992 Pretest and 1993 Main Survey of the 1993 National Mortality Followback Survey (NMFS), which will be conducted by NCHS, CDC. This survey will supplement information from death certificates in the vital statistics file with information on characteristics of the decedent. The pretest will examine approximately 800 deaths of individuals aged 15 years and over who died in 1992. The main survey will examine approximately 20,000 deaths of individuals aged 15 years and over who died in 1993. This will include 1,000 deaths to centenarians. Data will be collected by telephone and in-person interviews with informants listed on death certificates. Supplemental information will be collected by mail from health care facilities used by the decedent in the last year of life and from medical examiner/coroner records for referred deaths. NCHS, CDC will monitor data collection which, for the pretest, will be done by the U.S. Bureau of the Census.

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